REMARKS

I. Status Summary

The specification as been objected to for the presence of a typographical error on page 12, lines 26-27.

The Information Disclosure Statement (IDS) filed September 8, 2003 has been objected to as lacking a PTO-1449 form.

Claims 1-7, 9-16, 18-21, 23-27, 29-33, 36-59, 61, and 63 are pending in the present application. Claims 1-7, 9-12, 18, 24, 25, 29, 30, 33-38, 44, 49, 54, and 55 have been withdrawn by the United States Patent and Trademark Office (hereinafter "the Patent Office") Examiner upon the contention that the claims are drawn to an invention nonelected with traverse in Paper No. 17. Claims 13-16, 19-21, 23, 26, 27, 31, 32, 39-43, 45-48, 50-53, 56-59, 61, and 63 have been examined and presently stand rejected.

Claims 1-4, 9-12, 33, and 36-38 have been objected to as being mislabeled, the Patent Office asserting that these claims should be labeled "Withdrawn".

Claims 1, 13, 15, 18, 33, 39, 41, 44, 45, 49, 50, 56, and 63 have been objected to for the use of the term "harboring" or "harbouring".

Claims 15, 41, 46, 51, 56, and 63 have been objected to as unclear.

Claims 1-7, 9-12, 18, 24, 25, 29, 30, 33-38, 44, 49, 54, and 55 have been objected to as being drawn to a nonelected invention.

Claims 15, 16, 20, 21, 23, 27, 31, 32, 41, 42, 46, 47, 51, 52, 56-59, 61, and 63 have been rejected under 35 U.S.C. § 112, first paragraph, upon the contention that the specification, while being enabled for a method of treating restenosis or cancer by contacting the site of restenosis or cancer with a retrovirus encoding SDI-1 resulting in a therapeutic effect, does not reasonably provide enablement for using any mode of delivery as broadly claimed, using producer cells or capsules to treat disease, or using analogues or fragments of SDI-1 to treat disease.

Claims 13-16, 19-21, 23, 26, 27, 31, 32, 39-43, 45-48, 50-53, 59, 61, and 63 have been rejected under 35 U.S.C. § 112, second paragraph, upon several contention that the claims are indefinite.

Claims 13, 14, 19, 26, 27, 31, 32, 39, 40, 45, 48, 50, and 53 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Miller (1989, *Biotechniques* 7980-990; hereinafter "Miller") or Price (1987, *PNAS* 84:156-160; hereinafter "Price") in view of U.S. Patent No. 5,863,904 to Nabel (hereinafter "Nabel"). These claims have also been rejected under this section over Gunzburg (PCT International Patent Application WO 96/07748; hereinafter "Gunzburg") in view of Nabel.

Claims 32, 56, and 57 have been canceled. Claims 5-7, 12, 18, 24-25, 29, and 30 have been withdrawn from consideration. Claims 1, 3, 4, 9, 10, 13-15, 19, 21, 26, 27, 31, 33, 36, 37, 39-41, 43-46, 48-51, 53, 58, and 63 have been amended. Support for the amendments can be found throughout the specification as filed, including *inter alia* in the claims as filed. Additional support can be found on page 23, line 22 *et seq* (Example 3, "Construction of ProCon SDI retroviral vector") and Figure 7 (retroviral vector comprising in 5' to 3' order a 5' LTR, an SDI-1 coding sequence, and a 3' LTR) and page 8, lines 5-8, and original claims 27 and 28 (treatment of subjects having a tumor or restenosis).

Reconsideration of the application as amended and based on the remarks set forth herein below is respectfully requested.

II. Response to the Objection to the Specification

The specification has been objected to as containing a typographical error on page 12, lines 26-27. According to the Patent Office, the PCT International Patent Application Publication referred to is WO 96/07748, not WO 95/07748. Applicants have amended the specification to correct this typographical error.

Additionally, applicants have found that the "RELATED APPLICATIONS" section of the specification is incorrect. The instant application is a continuation of

PCT/EP96/04447, not PCT/US96/04447. Applicants have amended the specification appropriately.

III. Response to the Objection to the IDS

The Information Disclosure Statement (IDS) filed September 8, 2003 has been objected to as lacking a PTO-1449 form. A replacement PTO-1449 form has been produced and is enclosed herewith.

IV. Responses to the Claim Objections

Several claims have been objected to on various bases. After careful consideration of the objections and the Patent Office's bases therefor, applicants respectfully traverse the objections and submit the following remarks.

IV.A. Objection to Claims 1-4, 9-12, 33, and 36-38

Claims 1-4, 9-12, 33, and 36-38 have been objected to as being mislabeled, the Patent Office asserting that these claims should be labeled "Withdrawn". This objection appears to be based on the Patent Office's assertion in an Official Action dated May 14, 2002 that "the claims encompass retroviruses comprising RNA which is equivalent to the non-elected invention of retroviruses comprising antisense". *See* Official Action dated May 14, 2002 at page 2. Applicants specifically traverse the Patent Office's assertion that a recombinant retroviral particle, said particle comprising an RNA sequence which encodes SDI-1 as recited in claims 1 and 33, is equivalent to antisense and hence is drawn to a non-elected invention.

Applicants respectfully submit that the Patent Office has not articulated a scientifically sound basis for this assertion. The Patent Office appears to assume first that applicants are arguing that a retroviral RNA (*i.e.* the genome of the recombinant retroviral particles of claims 1 and 33) cannot encode SDI-1 because antisense is the non-coding strand used for mRNA synthesis. Applicants respectfully submit that this is not the basis of applicants' arguments, as explained in more detail as follows.

Applicants respectfully submit that claims 1 and 33 recite, *inter alia*, the following: "A method for producing a recombinant retroviral particle, said particle comprising an RNA sequence encoding an SDI-1 polypeptide...". Initially, applicants respectfully submit that the Patent Office is interpreting the term "encoding" in a way that is not entirely consistent with the meaning normally attached to the term by one of ordinary skill in the art. A strand that is "encoding" a polypeptide is the sense strand, even though the Patent Office is correct that the non-coding strand is the one that is used for mRNA transcription, and that this strand is typically regarded as the antisense strand. However, this argument does not suffice when one speaks of an mRNA itself, which is said to encode a protein. Given that mRNAs encode proteins, and mRNAs are sense strands, reference to a coding strand as an antisense strand is relevant only to double-stranded molecules, particularly double-stranded DNA molecules. Since claims 1 and 33 recite "an RNA sequence encoding an SDI-1 polypeptide", such a reference is inapplicable.

This distinction is particularly critical when one considers the nature of the RNA molecule recited in claims 1 and 33. The RNA molecule that encodes SDI-1 in claims 1 and 33 is an RNA molecule present within a retroviral particle. As is known in the art, retroviruses have single-stranded RNA genomes, and in retroviruses, these RNA molecules are plus strand (that is, sense strand) molecules. Given that claims 1 and 33 recite an RNA molecule that is present within a retroviral particle, and this strand necessarily must be a single-stranded plus strand, there is no scientific basis for asserting that the RNA sequence present within the presently claimed particles can be an antisense strand.

Applicants respectfully submit that the Restriction Requirement was based on the use of a DNA encoding SDI-1 versus antisense SDI-1 molecules, and the claimed RNA molecules are not antisense molecules. Applicants further respectfully submit that the RNA molecules present within the instantly claimed particles are the natural and logical vehicles for employing the retroviral vectors of the presently claimed

subject matter in the method of treatment claims exemplified by claim 27, and thus fall within the scope of elected Group I rather than nonelected Group II.

Accordingly, applicants respectfully submit that the Patent Office's assertion that claims 1-4, 9-12, 33, and 36-38 were constructively withdrawn as a result of the election in response to the Restriction Requirement is improper, and applicants respectfully request that the objection be withdrawn and that these claims be rejoined and examined.

IV.B. Objection to Claims 1, 13, 15, 18, 33, 39, 41, 44, 45, 49, 50, 56, and 63

Claims 1, 13, 15, 18, 33, 39, 41, 44, 45, 49, 50, 56, and 63 have been objected to for the use of the term "harboring" or "harbouring". Applicants respectfully traverse this objection and submit the following comments.

Initially, applicants respectfully submit that the Patent Office has not articulated any reason for the instant objection, suggesting only that the objected to term should be changed to "comprising". Thus, it is not clear why the Patent Office disapproves of the use of these terms regarding a producer cell line that "harbors" a DNA construct, particularly in view of the fact that the Patent Office has clearly correctly interpreted "harboring" and "comprising" as synonymous.

However, in an effort to facilitate the prosecution of the pending claims, applicants have amended all occurrences of the terms "harboring" and "harbouring" to "comprising" as recommended by the Patent Office in the pending claims. Applicants respectfully submit, however, that these amendments are solely for the purposes of clarity, and are not to be construed as a surrender of any subject matter encompassed by the claims as previously presented.

Accordingly, applicants respectfully submit that the objection has been addressed. Claim 56 has been canceled, and thus the objection is believed to be moot as to this claim. Thus, applicants respectfully request that the objection of claims 1, 13, 15, 18, 33, 39, 41, 44, 45, 49, 50, and 63 be withdrawn and the claims allowed at this time.

IV.C. Objection to Claims 15, 41, 46, 51, 56, and 63

Claims 15, 41, 46, 51, 56, and 63 have been objected to as unclear. Particularly, the Patent Office has objected to the language of the preambles and to the phrase "said capsule comprising a porous capsule wall being permeable to the retroviral particles produced by said producer cell". While it is not clear why the particular language employed is objectionable, in an effort to facilitate the prosecution of the pending claims, applicants have amended the claims as suggested by the Patent Office. Applicants respectfully submit, however, that these amendments are solely for the purposes of clarity, and are not to be construed as a surrender of any subject matter encompassed by the claims as previously presented.

Accordingly, applicants respectfully submit that the objection has been addressed. Claim 56 has been canceled, and thus the objection is believed to be moot as to this claim. Thus, applicants respectfully request that the objection of claims 15, 41, 46, 51, and 63 be withdrawn and the claims allowed at this time.

IV.D. Objection to Claims 1-7, 9-12, 18, 24, 25, 29, 30, 33-38, 44, 49, 54, and 55

Claims 1-7, 9-12, 18, 24, 25, 29, 30, 33-38, 44, 49, 54, and 55 have been objected to as being drawn to a nonelected invention. The basis for this objection is similar to that discussed hereinabove for the objection to claims 1-4, 9-12, 33, and 36-38. Additionally, the Patent Office asserts that claims 5-7, 18, and 24 explicitly require the DNA have antisense. After careful consideration of the objection and the Patent Office's bases therefor, applicants respectfully traverse the objection and submit the following remarks.

Initially, applicants respectfully submit that claims 5-7, 18, 24, 25, 29, and 30 have been withdrawn from consideration. Thus, applicants respectfully submit that the instant objection to these claims has been addressed.

Applicants respectfully submit that the Patent Office's assertion that claims 1-4, 9-12, 33-38, 44, 49, 54, and 55 encompass an antisense strategy is unsupported as discussed hereinabove with reference to the objection to claims 1-4, 9-12, 33, and 36-

38 (incorporated herein by reference). Summarily, since retroviral particles have single-stranded, sense strand genomes, it cannot be said that the recombinant retroviral particles of claims 1-4, 9-12, 33-38, 44, 49, 54, and 55 encompass an SDI-1 antisense approach. This is particularly true in view of the statements of record by applicants that SDI-1 antisense approaches would be expected to stimulate cell proliferation, and claims 1, 33, 44, and 49, the independent claims from which all of the instantly objected to claims depend, all recite that the RNA sequence encodes a polypeptide that inhibits cell proliferation. Thus, applicants respectfully submit that the objected to claims clearly do not involve an SDI-1 antisense strategy.

Accordingly, applicants respectfully submit that the objection of claims 1-7, 9-12, 18, 24, 25, 29, 30, 33-38, 44, 49, 54, and 55 as being drawn to a nonelected invention has been addressed. Claims 5-7, 18, 24, 25, 29, and 30 have been withdrawn. Claim 12 was withdrawn in the previously filed Amendment dated September 5, 2003. Thus, it is believed that the objection to these claims is moot. Thus, applicants respectfully request that the objection of claims 1-4, 9-11, 33-38, 44, 49, 54, and 55 be withdrawn, and that the claims be allowed at this time.

V. Claim Rejection under 35 U.S.C. § 112, First Paragraph

Claims 15, 16, 20, 21, 23, 27, 31, 32, 41, 42, 46, 47, 51, 52, 56-59, 61, and 63 have been rejected under 35 U.S.C. § 112, first paragraph, upon the contention that the specification, while being enabled for a method of treating restenosis or cancer by contacting the site of restenosis or cancer with a retrovirus encoding SDI-1 resulting in a therapeutic effect, does not reasonably provide enablement for using any mode of delivery as broadly claimed, using producer cells or capsules to treat disease, or using analogues or fragments of SDI-1 to treat disease. After careful consideration of the rejection and the Patent Office's basis therefor, applicants respectfully traverse the rejection and submit the following remarks.

V.A. The Rejection of Claims 21, 26, 27, 32, 59, and 63

The Patent Office asserts that claims 21, 26, 27, 32, 59, and 63 are directed toward treating disease using a retrovirus, a producer cell that makes a retrovirus, or an encapsulated producer cell that makes a retrovirus encoding SDI-1, and that these claims encompass any route of administration. The Patent Office contends that the specification as filed does not enable treating disease, specifically restenosis or cancer, using any route of administration as broadly claimed. Accordingly to the Patent Office, the art at the time of filing did not teach how to treat diseases responsive to the anti-proliferative activity of SDI-1 using a retrovirus encoding SDI-1 other than by administering the virus directly to the site of disease.

Thus, it appears that the first aspect of the instant rejection concerns the mode of administration. Initially, applicants respectfully submit that claim 26 recites a method for introducing DNA into human cells *in vitro*. It does not involve administering the retrovirus to a subject, and thus the instant rejection is inapplicable to claim 26. Applicants request that the rejection be withdrawn as to claim 26. Additionally, applicants note that Claim 32 has been canceled, and thus the rejection is moot as to this claim.

Thus, turning now to claims 21, 27, 59, and 63, claim 27 recites a method for treating a subject having a tumor or restenosis, the method comprising administering to the subject a therapeutically effective amount of a recombinant retroviral particle produced by the isolated producer cell line of claim 13 at the site of the tumor or restenosis. The Patent Office has conceded that at the time of filing the art taught how to treat diseases responsive to the anti-proliferative activity of SDI-1 using a retrovirus encoding SDI-1 by administering the virus directly to the site of disease (*see* Official Action at page 5). Thus, applicants respectfully submit that the isolated producer cell line of claim 13 produces a retrovirus encoding an SDI-1 polypeptide or a functional fragment thereof; *i.e.* a retrovirus encoding an SDI-1 polypeptide with anti-proliferative activity. Given that claim 27 therefore recites delivering this retrovirus to the site of the tumor or restenosis, applicants respectfully submit that the instant specification

provides a fully enabled disclosure of claim 27. Applicants respectfully request that the instant rejection be withdrawn as to claim 27.

Continuing with the instant rejection, applicants respectfully submit that claims 21, 59, and 63 recite *inter alia* methods for treating a tumor or restenosis in an individual (or a subject) by administering a capsule comprising an SDI-1 retrovirus producer cell to a site of the tumor or the restenosis. Applicants respectfully submit that the Patent Office concedes that administering the virus directly yo the site of disease is enabled by the specification of the instant application. Accordingly, the instant rejection appears to be based on the following assertions by the Patent Office: that the art did not teach how to administer the capsules to treat disease, and that the art did not teach that the capsules could produce adequate amounts of retrovirus such that a therapeutic effect could be obtained. The Patent Office thus contends that it would require undue experimentation to determine how to administer capsules to treat disease. Applicants respectfully traverse these assertions and submit the following remarks.

Applicants respectfully submit that the instant specification teaches one of ordinary skill in the art how to generate cell lines that produce SDI-1 encoding retroviruses, how to encapsulate those cell lines, and how to administer those capsules to subjects having tumors or restenosis in order to treat their diseases. The Patent Office, on the other hand, relies instead on conclusory statements that do not appear to be supported by any scientific evidence. It would appear that the Patent Office would require applicants to provide specific working examples of every technique disclosed in the instant application. While the presence or absence of working examples is one consideration in the overall evaluation of enablement, working examples are not required under 35 U.S.C. §112, first paragraph, to comply with the enablement standard presented therein. Indeed, the M.P.E.P. states that the specification need not contain an example if the invention is otherwise disclosed in such a manner that one skilled in the art will be able to practice it without an undue amount of experimentation. M.P.E.P. §2164.02. The M.P.E.P. also states that a lack

of working examples or lack of evidence that the claimed invention works as described should never be the sole reason for rejecting the claimed invention on the grounds of lack of enablement. Id.

However, applicants respectfully submit that this is exactly what the Patent Office is now doing: summarily concluding that the specification is non-enabling solely because, in essence, the Patent Office believes that there are no working examples of the presently claimed subject matter disclosed in the present U.S. patent application. This appears to be the basis of the instant rejection despite the specification teaching that the transfer and expression of an SDI-1 coding sequence inhibits cell proliferation, the knowledge in the art that retroviruses can be used to transfer coding sequences to cells, and the disclosure in the present U.S. patent application that encapsulated producer cells can produce sufficient retrovirus to infect cells in the vicinity of the capsule. The Patent Office offers no scientific evidence that would lead one of skill in the art to conclude that undue experimentation would be required to practice the instant claims. Accordingly, applicants respectfully submit that the instant specification provides an enabling disclosure with respect to claims 21, 59, and 63, and respectfully request that the rejection of these claims under § 112, first paragraph, be withdrawn at this time.

Applicants further respectfully submit that the data presented in applicants' U.S. Patent Application Serial No. 08/996,460 (the U.S. National Stage Application corresponding to WO 97/01357) represents data on which applicants can rely to support the contention that one of ordinary skill in the art could practice the presently claimed subject matter without undue experimentation. The '460 Application, now U.S. Patent No. 6,776,985, demonstrates that encapsulated producer cells can be implanted in a target and cause retroviral infection and gene expression in cells in the vicinity of the target.

Summarily, applicants respectfully submit that the specification satisfies the requirements of 35 U.S.C. § 112, first paragraph, with regard to present claims 21, 26, 27, 32, 59, and 63. Claim 32 has been canceled, and thus the rejection is believed to

be moot as to this claim. Thus, applicants respectfully request that the rejection of claims 21, 26, 27, 59, and 63 be withdrawn and the claims allowed at this time.

V.B. The Rejection of Claim 31

The Patent Office asserts that claim 31 is limited to injecting a retroviral particle "at a site of the tumor", but is not limited to treating patients having tumors. With regard to the instant rejection, applicants respectfully submit that claim 27, the claims from which claim 31 depends, clearly recites a method for the treatment of a tumor or restenosis comprising administering to a living animal body, including a human, in need thereof... . Thus, applicants respectfully submit that a method of treatment of a tumor or restenosis of a living animal body, including a human in need thereof is a living animal body, including a human, having a tumor or restenosis. Thus, it is not necessary to explicitly recite that which is implicitly disclosed in the claim in view of claim 31's dependency from claim 27.

Nonetheless, in an effort to facilitate prosecution of the pending claims, applicants have amended claim 27, the claim from which claim 31 depends, to recite a method for treatment of a subject having a tumor or restenosis. Accordingly, applicants respectfully submit that this aspect of the instant rejection as been addressed, and respectfully request that it be withdrawn.

V.C. The Rejection of Claims 15, 16, 20, 21, 23, 41, 42, 46, 47, 51, 52, 56-59, 61, and 63

Claims 15, 16, 20, 21, 23, 41, 42, 46, 47, 51, 52, 56-59, 61, and 63 have been rejected under this section upon the assertion that the specification as filed does not provide enablement of the use of producer cells or capsules comprising producer cells to treat disease. According to the Patent Office, the art at the time of filing did not teach how to administer a producer cell or an encapsulated producer cell making retrovirus to treat disease or that producer cells or encapsulated producer cells provide adequate amounts of retrovirus such that a therapeutic effect could be obtained. The Patent Office further asserts that the specification does not provide adequate guidance regarding the site of administration of producer cells or capsules or the secretion of

retrovirus from the producer cells or capsules that correlates to the amount of retrovirus directly injected to the site of disease that is therapeutic. As a result, the Patent Office contends that given the unpredictability in the art taken with the guidance provided, it would require one of ordinary skill in the art undue experimentation to determine how to administer producer cells or capsules to treat disease. After careful consideration of the rejection and the Patent Office's bases therefor, applicants respectfully traverse the rejection and submit the following remarks.

Applicants initially submit that claims 56 and 57 have been canceled as duplicative of claims 41 and 42, and thus the rejection is moot as to these claims. Furthermore, applicant respectfully submit that the remaining claims recite *inter alia* the administration of producer cells present within capsules, and thus are not directed to the administration of producer cells themselves.

In response to the instant rejection, applicants direct the Patent Office's attention to the remarks presented in the previous section. Applicants respectfully submit that these remarks are equally applicable to the instant rejection, and thus address the instant rejection. Summarily, applicants respectfully submit that the instant specification clearly demonstrates that encapsulated producer cells can be implanted in a target and cause retroviral infection and gene expression in cells in the vicinity of the target. Applicants further respectfully submit that the introduction of an SDI-1 coding sequence into these cells will inhibit their proliferation, and thus treat either a tumor or restenosis.

With regard to the contention that the specification and/or art did not teach "the secretion of retrovirus from the producer cells or capsules that correlates to the amount of retrovirus directly injected to the site of disease that is therapeutic", applicants respectfully submit that there is no requirement that applicants demonstrate a correlation between an amount of retrovirus that can be delivered by direct injection and an amount of retrovirus that is delivered by administration of the claimed capsules. Rather, applicants respectfully submit that they have taught administration of encapsulated producer cells that generate recombinant retroviruses that when

implanted cause the retrovirus to infect cells in the vicinity. This infection transfers an SDI-1 coding sequence to the cell, where it is expressed. The specification further teaches that expression of a retrovirally-encoded SDI-1 sequence causes inhibition of proliferation of the cell. Taken together, these disclosures clearly suggest to one of skill in the art that encapsulated producer cells can be used to treat the claimed conditions because inhibited proliferation that results from the infection of a tumor or vascular smooth muscle cell with the retrovirus would decrease the extent of tumor growth or restenosis.

Summarily, applicants respectfully submit that the specification satisfies the requirements of 35 U.S.C. § 112, first paragraph, with regard to the present claims 15, 16, 20, 21, 23, 41, 42, 46, 47, 51, 52, 56-59, 61, and 63. Claims 56 and 57 have been canceled, and thus the rejection is believed to be moot as to these claims. Thus, applicants respectfully request that the rejection of claims 15, 16, 20, 21, 23, 41, 42, 46, 47, 51, 52, 58-59, 61, and 63 be withdrawn and that the claims allowed at this time.

V.D. The Rejection of Claims 15, 46, 47, 51, 52, and 63

Claims 15, 46, 47, 51, 52, and 63 have been rejected under 35 U.S.C. § 112, first paragraph, on the contention that the specification as filed does not enable functional fragments of SDI-1. According to the Patent Office,

> Applicants have not provided the amount of inhibition required for a fragment *in vitro* that indicates the fragment is capable of treating disease. Applicants have not provided any data indicating any fragment has the same function as full length SDI-1 [in] such an assay. Without such guidance, it would require one of skill undue experimentation to determine any fragment or analogue of SDI-1 capable of treating disease *in vivo*. It would require one of skill undue experimentation to determine whether a retrovirus encoding amino acids 1-71 or 42-58 of human SDI-1 using any route of administration as broadly claimed would have a therapeutic effect. It cannot be determined whether amino acids 1-71 and 42-58 of the SDI-1 protein as described by El-Deiry, Harper or Xiong have the same antiproliferative activity of full length SDI-1.

Official Action at page 8 (emphasis supplied; quoting the Official Action dated May 14, 2002). After careful consideration of the rejection and the Patent Office's bases therefor, applicants respectfully traverse the rejection and submit the following remarks.

Applicants respectfully submit that the Patent Office has not satisfied its burden of presenting a *prima facie* case of non-enablement simply by offering conclusory statements that applicants have not demonstrated the ability of fragments to produce a therapeutic effect *in vivo*. Applicants submit that the specification as filed clearly discloses that the human SDI-1 polypeptide is 164 amino acids long, and that deletion of amino acids 72-164 does not significantly effect the inhibitory activity of the polypeptide. *See* Specification at page 8, lines 22-25. The unmistakable conclusion to be drawn from this statement is that a polypeptide comprising amino acids 1-71 of human SDI-1 has an insignificantly different level of inhibitory activity when compared to that of the full length human SDI-1.

The Patent Office offers no competent scientific evidence to contradict this statement. According to the Training Materials for Examining Patent Applications with Respect to 35 U.S.C. Section 112, First Paragraph-Enablement in Chemical/Biotechnical Applications (hereinafter "The Training Materials"), "without a reason to doubt the truth of the statements made in the patent application, the application must be considered enabling" (emphasis added; citing *In re Wright*, 999 F.2d 1557, 1563 (Fed. Cir. 1993) and *In re Marzocchi*, 439 F.2d 220, 223 (CCPA 1971)). Applicants respectfully submit that no such reason has been articulated by the Patent Office. On the contrary, the specification as filed clearly teaches that infection of EJ human bladder carcinoma cells with a retrovirus encoding SDI-1 results in inhibition of proliferation of the cells *in vitro*. Accordingly, the specification clearly discloses that a polypeptide comprising amino acids 1-71 of human SDI-1 would also be expected to inhibit proliferation of tumor cells.

The Patent Office appears to be attempting to require that applicants produce specific evidence of SDI-1 fragments that have been shown capable of treating

disease *in vivo*. Applicants respectfully submit that production of such evidence is not required in order to show enablement even if the Patent Office had successfully presented a *prima facie* case of non-enablement, which it has not. Applicants have disclosed that SDI-1 delivered by a retrovirus inhibits proliferation of tumor cells. Applicants have further disclosed that amino acids 1-71 of human SDI-1 also have this activity. This contention is supported by Nakanishi *et al.* of record (14 *EMBO J* 555-563, 1995), which clearly indicates that an SDI-1 derivative consisting of amino acids 1-71 inhibits DNA synthesis virtually identically to the full length SDI-1 (*see* Figure 1). Nakanishi of record also discloses that SDI-1 is a cyclin-dependent kinase (cdk) inhibitor. As such, it works in a cell autonomous fashion to inhibit cyclins, which are necessary for DNA synthesis and cell division. Thus, applicants respectfully submit that there is nothing in the nature of the protein's activity that would lead one of skill in the art to conclude that *in vitro* activity would not correlate with *in vivo* activity. Accordingly, applicants respectfully submit that the Patent Office has not articulated any basis for concluding that a polypeptide comprising amino acids 1-71 of human SDI-1 would not have the same antiproliferative activity of full length SDI-1.

Summarily, the Patent Office has presented a series of conclusory statements regarding the alleged deficiencies of the enablement of the specification. These statements are contrary to the teachings of the art, and have not been supplemented by any sound scientific rationale whatsoever. Accordingly, applicants respectfully submit that the Patent Office has not overcome the presumption that the specification as filed is enabling, and have thus not presented a *prima facie* case of non-enablement. Applicants respectfully request that the rejection of claims 15, 46, 47, 51, 52, and 63 under 35 U.S.C. § 112, first paragraph, be withdrawn, and that the claims be allowed at this time.

VI. Claim Rejections under 35 U.S.C. § 112, Second Paragraph

Claims 13-16, 19-21, 23, 26, 27, 31, 32, 39-43, 45-48, 50-53, 59, 61, and 63 have been rejected under 35 U.S.C. § 112, second paragraph, on several bases.

After careful consideration of the rejections and the Patent Office's bases therefor, applicants respectfully traverse the rejections and submit the following remarks.

There are several rejections under this section, which are summarized as follows:

Claims	Objected to Term or Phrase
13, 15, 39, 45, 50	"a 5' LTR region of the structure U3-R-U5"
13, 15, 39, 45, 50	"one or more sequences selected from coding and noncoding sequences"
13, 15, 39, 45, 50	"a 3' LTR region comprising a completely or partially deleted U3 region wherein said deleted U3 region is replaced by a polylinker sequence containing a regulatory element or a promoter, followed by the U5 and R region, characterized in that at least one of the coding sequences is a sequence encoding SDI-1" (6 embedded rejections)
13, 15, 39, 45, 50	"said SDI-1 sequence encoding a polypeptide with SDI-1 activity of inhibiting cell proliferation"
13, 15, 39, 45, 50	"a polypeptide with SDI-1 activity of inhibiting cell proliferation and being under transcriptional control of said regulatory element or promoter"
15	"which encapsulates"
21, 27, 31, 59, 63	"the site of the tumor"
27, 31, 63	"the living animal body"
32	"administering a producer cell line according to claim 13 to the site of tumor or restenosis"
32	"the site of tumor or restenosis"
63	"a capsule"; "a porous capsule wall"; "core"

VI.A. Rejections of Claims 13, 15, 39, 45, 50

The Phrase "A 5' LTR Region of the Structure U3-R-U5"

The Patent Office has rejected claims 13, 15, 39, 45, 50 upon the contention that the phrase "a 5' LTR region of the structure U3-R-U5" is indefinite. According to the Patent Office, one of ordinary skill in the art would not know when the phrase had been met because it cannot be determined how much of the U3, R, or U5 region is required to have the structure of a U3, R, or U5 region. After careful consideration of

the rejection and the Patent Office's basis therefor, applicants respectfully traverse the rejection and submit the following remarks.

Initially, applicants respectfully submit that claim 15 does not employ the objected to phrase, nor does it depend from a claim that does. Thus, applicants respectfully submit that the rejection is inapplicable to claim 15, and request its withdrawal as to this claim.

Continuing with the instant rejection, applicants respectfully submit that claims 13, 39, 45, and 50 all recite the objected to phrase in the following context: a retroviral vector comprising in 5' to 3' order a 5' LTR region of the structure U3-R-U5. Thus, this element simply recites a retroviral vector with a 5' LTR. The Patent Office has not suggested any reason why one of ordinary skill in the art would not understand the metes and bounds of this claim element. Applicants respectfully submit that one of ordinary skill in the art would recognize that retroviral vectors have 5' LTRs, and that the 5' LTR is characterized by three discrete regions known as the U3, R, and U5 regions. Given that the claims must be interpreted from the perspective of the skilled artisan, applicants respectfully submit that the phrase "a 5' LTR region of the structure U3-R-U5" does not render the claims indefinite as the phrase simply recites the well known features of a retroviral LTR.

Furthermore, applicants respectfully submit that other United States Patents employ the same general language. For example, claim 18 of U.S. Patent No. 6,730,511 recites "A recombinant retroviral vector which is capable of undergoing promoter conversion and is replication-defective comprising, in operable linkage, a) a 5' long terminal repeat region comprising the structure U3-R-U5...". Similarly, claim 6 of U.S. Patent No. 6,117,681 recites "The packaging cell line according to claim 5 wherein the murine leukemia virus based retroviral vector comprises, in operable linkage: a) a 5' LTR region originating from murine leukemia virus and of the structure U3-R-U5...". Clearly, and contrary to the Patent Office's assertions, the phrase "a 5' LTR region of the structure U3-R-U5" does not render claims 13, 39, 45, and 50 indefinite.

Accordingly, applicants respectfully submit that the rejection of claims 13, 15, 39, 45, and 50 has been addressed. Applicants respectfully request that the instant rejection be withdrawn and the claims allowed at this time.

The Phrase "One or More Sequences Selected from Coding and Noncoding Sequences"

Claims 13, 15, 39, 45, 50 have also been rejected under this section upon the contention that the phrase "one or more sequences selected from coding and noncoding sequences" renders the claims indefinite. According to the Patent Office, it cannot be determined what is being excluded or included by this limitation because the only two types of nucleic acid sequences are coding and noncoding sequences.

Initially, applicants respectfully submit that claim 15 does not employ the objected to phrase, nor does it depend from a claim that does. Thus, applicants respectfully submit that the rejection is inapplicable to claim 15, and request its withdrawal as to this claim.

Continuing with the instant rejection, applicants respectfully submit that claims 13, 39, 45, and 50 have been amended to delete the objected to phrase. The relevant sections of these claims now recite *inter alia* "a sequence encoding" a polypeptide, an SDI-1 polypeptide, etc. Thus, the claims now clearly recite what is being included by this element. Applicants respectfully submit that the amendments to these claims are made solely for the purpose of clarity, and are not to be interpreted as a surrender of any subject matter encompassed by the claims as previously presented.

Accordingly, applicants respectfully submit that the instant rejection of claims 13, 15, 39, 45, and 50 have been addressed. Applicants respectfully request that the instant rejection be withdrawn and the claims allowed at this time.

The Phrase "A 3' LTR Region Comprising a Completely or Partially Deleted U3 Region..."

Claims 13, 15, 39, 45, 50 have also been rejected under this section on several different basis related to the phrase "a 3' LTR region comprising a completely or partially deleted U3 region wherein said deleted U3 region is replaced by a polylinker

sequence containing a regulatory element or a promoter, followed by the U5 and R region, characterized in that at least one of the coding sequences is a sequence encoding SDI-1". After careful consideration of these rejections and the Patent Office's bases therefor, applicants respectfully traverse the rejections and submit the following remarks.

Initially, applicants respectfully submit that claim 15 does not employ the objected to phrase, nor does it depend from a claim that does. Thus, applicants respectfully submit that the rejection is inapplicable to claim 15, and request its withdrawal as to this claim.

The first aspect of the instant rejection relates to the contention that "the structure of what applicants consider a complete or partial deletion of the U3 region cannot be determined". However, the Patent Office has presented no basis for why one of ordinary skill in the art would not understand this phrase. Those of skill know that retroviral vectors typically have 3' LTRs. They also know that the 3' LTR comprises the structure U3-R-U5. Also, retroviral vectors are typically designed based on known retroviruses, such as Moloney Murine Leukemia Virus. As such, applicants respectfully submit that the nucleic acid sequences of retroviral vectors always have a context upon which the skilled artisan can judge whether or not the 3' LTR U3 sequence is present and/or contains a complete or partial deletion.

Applicants further respectfully submit that the instantly objected to phrase is being viewed in exclusion and that when the phrase is viewed in the context of the whole claim, it is clear that the claim recites *inter alia* a retroviral vector comprising a 5' LTR, an SDI-1 coding sequence, and a 3' LTR in which the U3 region of the 3' LTR is incomplete or absent. Given that all of these structures are recognizable by the skilled artisan, there is no basis for concluding that the skilled artisan would not understand the metes and bounds of the element.

Continuing with the instant rejection, the Patent Office also asserts that the metes and bounds of sequences encompassed by the phrase "polylinker sequence containing a regulatory element or promoter" cannot be determined. The Patent Office

also asserts that it cannot be determined if polylinkers encompass restriction sites, splice donors, splice acceptors, promoters and polyadenylation signals or if polylinkers are limited to restriction sites. And finally, the Patent Office asserts that it cannot be determined if a "polylinker sequence containing a regulatory element or a promoter" has a polylinker and a regulatory element/promoter or if the polylinker is the regulatory element/promoter.

With respect to the instant rejection, applicants respectfully submit that the claims recite *inter alia* a retroviral vector into which a heterologous sequence comprising a regulatory element has been cloned into a completely or partially deleted U3 region of the 3' LTR. Thus, there is no practical difference to the various "alternatives" that the Patent Office alleges can be encompassed by the claim element.

However, in an effort to facilitate the prosecution of the instant claims, applicants have amended subsection (c) of claims 13, 39, 45, and 50 to recite a 3' LTR region comprising a completely or partially deleted U3 region, wherein into said deleted U3 region has been cloned a polylinker sequence into which a regulatory element or a promoter has been inserted. Applicants respectfully submit that these amendments address the instant rejection.

Claims 13, 15, 39, 45, and 50 have also been rejected on the contention that the phrase "followed by" is unclear. claim 15 does not employ the objected to phrase, nor does it depend from a claim that does. Thus, applicants respectfully submit that the rejection is inapplicable to claim 15, and request its withdrawal as to this claim. Applicants have amended claims 13, 39, 45, and 50 to recite *inter alia* an isolated producer lines stably transfected with a retroviral vector comprising in 5' to 3' order (a) a 5' LTR...; (b) a sequence encoding a polypeptide...; and (c) a 3' LTR... . Applicants respectfully submit that the amended claim clearly recite that the retroviral vector has an intact 5' LTR, a coding sequence cloned into the body of the vector, and a 3' LTR having a complete or partial deletion of the U3 region. As one of ordinary skill in the art would recognize, the 3' LTR of a retroviral vector has the structure U3-R-U5, and

thus the structure of the 3' LTR used to stably transfect the producer cell line has the general structure U3*-R-U5, wherein U3* corresponds to the modified U3 region.

Further, the phrase "followed by the U5 and R region" has been deleted from the claims 13, 39, 45, and 50, as well as claims 1 and 33. Accordingly, applicants respectfully submit that the instant rejection of claims 13, 15, 39, 45, and 50 has thus been addressed.

Summarily, applicants respectfully submit that the instant rejections of claims 13, 15, 39, 45, and 50 have been addressed, and that the claims are in condition for allowance. Applicants respectfully request that the instant rejections be withdrawn and the claims allowed at this time.

The Phrase "Said SDI-1 Sequence Encoding a Polypeptide with SDI-1 Activity of Inhibiting Cell Proliferation"

Claims 13, 15, 39, 45, and 50 have been rejected on the contention that the above quoted phrase lacks antecedent basis in the claims. Applicants have deleted the phrase from claims 13, 39, 45, and 50. Furthermore, applicants respectfully submit that the objected to phrase does not appear in claim 15.

Accordingly, applicants respectfully submit that the instant rejection of claims 13, 15, 39, 45, and 50 has been addressed, and that the claims are in condition for allowance. Applicants respectfully request that the instant rejection be withdrawn and the claims allowed at this time.

The Phrase "A Polypeptide with SDI-1 Activity of Inhibiting Cell Proliferation and Being Under Control of Said Regulatory Element or Promoter"

Claims 13, 15, 39, 45, and 50 have been rejected on the contention that the above quoted phrase renders the claims indefinite. According to the Patent Office, it is unclear whether applicants are attempting to ascribe the function of SDI-1 to a particular activity or whether the nucleic acid sequence encoding SDI-1 is under transcriptional control of the regulatory element/promoter.

Initially, applicants respectfully submit that claim 15 does not employ the objected to phrase, nor does it depend from a claim that does. Thus, applicants

respectfully submit that the rejection is inapplicable to claim 15, and request its withdrawal as to this claim.

Continuing with the instant rejection, applicants respectfully submit that claims 13, 39, 45, and 50 have been amended to recite *inter alia* the following:

An isolated producer cell line stably transfected with a retroviral vector comprising in 5' to 3' order:

(a) a 5' LTR region of the structure U3-R-U5;

(b) a sequence encoding an SDI-1 polypeptide (or a functional fragment thereof); and

(c) a 3' LTR region comprising a completely or partially deleted U3 region, wherein into said deleted U3 region has been cloned a polylinker sequence into which a regulatory element or a promoter has been inserted,

wherein said SDI-1 polypeptide inhibits cell proliferation, and said isolated producer cell line comprises at least one DNA construct encoding a protein required for said retroviral vector to be packaged.

Applicants respectfully submit that the claimed producer cell lines comprise a retroviral vector and produce retroviruses that upon infection of a target cell cause the sequence encoding the SDI-1 polypeptide to come under transcriptional control of the regulatory element or promoter that has been inserted into the polylinker present in the U3 deletion. This is a principle of operation of the ProCon vectors disclosed in the instant specification: namely, that a coding sequence (for example, a sequence encoding an SDI-1 polypeptide or a functional fragment thereof) is cloned into the body of a retroviral vector, which after infection of a target cell becomes operatively linked to a heterologous promoter that has been inserted into a completely or partially deleted U3 region of the 3' LTR as a result of the reverse transcription of the retroviral genome that causes the 3' U3 region to be duplicated in the 5' LTR. Thus, applicants cannot adopt the suggestion of the Patent Office to amend the claims to recite "a vector comprising a nucleic acid sequence encoding SDI-1 operably linked to a

promoter" because the vector and the nucleic acid sequence encoding the SDI-1 polypeptide or functional fragment thereof are intentionally not operably linked in the retroviral vector used to transfect the producer cell line.

Nonetheless, applicants respectfully submit that as a result of the amendments to claims 13, 39, 45, and 50, the instant rejection of claims 13, 15, 39, 45, and 50 has been addressed. Applicants respectfully request that the instant rejection be withdrawn and the claims allowed at this time.

The Phrase "Which Encapsulates"

Claim 15 has been rejected upon the contention that the phrase "which encapsulates" renders the claim indefinite. Applicants have amended the claim to recite a capsule comprising, which the Patent Office has indicated would overcome the instant rejection.

Accordingly, applicants respectfully submit that the instant rejection of claim 15 has been addressed. Applicants further respectfully submit that as a result of the amendments and remarks presented hereinabove, claims 13, 15, 39, 45, and 50 are in condition for allowance. Applicants respectfully request that the rejections under 35 U.S.C. § 112, second paragraph, be withdrawn and the claims allowed at this time.

VI.B. Rejections of Claims 21, 27, 31, 59, 63

Claims 21, 27, 31, 59, 63 have been rejected under 35 U.S.C. § 112, second paragraph, upon several contentions. After careful consideration of the rejections and the Patent Office's bases therefor, applicants respectfully traverse the rejections and submit the following remarks.

The first basis for the instant rejection is the assertion that the claims do not require the "individual" have a tumor or restenosis. Initially, applicants respectfully submit that the claims recite methods of treating a tumor or restenosis in an individual, and thus clearly indicate that the individual must have a tumor or restenosis. Applicants respectfully submit that the Patent Office has offered no basis for concluding that the claim is indefinite. Applicants respectfully submit that is it implicit in the claims that treating a tumor or restenosis in an individual by administering a

composition to the individual at a site of the tumor or restenosis that the individual have a tumor or restenosis.

Nonetheless, in an effort to facilitate the prosecution of the instant claims, claims 21, 27, 59, and 63 have been amended to recite that the individual (or subject) has a tumor or restenosis. Applicants respectfully submit, however, that these amendments are solely for the purposes of clarity, and are not to be construed as a surrender of any subject matter encompassed by the claims as previously presented.

The Patent Office also asserts that the particles may be administered to patients with cancerous blood cells that do not have a "site" as claimed, and that according to the specification, treating metastases is part of the invention, but it is unclear if a metastasis "site" is the organ in which a metastasis is found or the cancerous tissue itself. With respect to the instant assertion, applicants respectfully submit that when the disclosure is understood in its entirety, it is clear that the administration of either the claimed capsules or recombinant retroviral particles is to a site that would allow the recombinant retroviral particles to infect the cells of interest (*e.g.* tumor cells or the cells involved in the restenosis). Applicants respectfully submit that "at the site" is thus defined to encompass any site that would allow this infection to take place. Furthermore, one of ordinary skill in the art would recognize how to administer the capsules/retroviruses in such a way as to promote the infection of the relevant cells upon a review of the disclosure of the present U.S. patent application as filed. Thus, given the nature of the claims considered in the context of the invention as a whole, applicants respectfully submit that the phrase "at the site of the tumor" is not indefinite.

Continuing with the instant rejection, the Patent Office also asserts that the phrase "at the site" in claims 21, 27, 31, 59, 63 is unclear because the Patent Office is not sure if "at the site" is limited to administering the composition directly into the tumor or restenosis or if the phrase encompasses any means that cause administration to the site of the tumor or restenosis.

Applicants respectfully submit that the claims at issue recite administering a retroviral particle or a capsule at a site of the tumor or the restenosis. As such and as indicated hereinabove, applicants respectfully submit that "at the site" is thus defined to encompass any site that would allow this infection to take place. Furthermore, one of ordinary skill in the art would recognize how to administer the capsules/retroviruses in such a way as to promote the infection of the relevant cells upon a review of the disclosure of the present U.S. patent application as filed.

Finally, applicants respectfully submit that the phrase "at a site of the tumor" is employed in numerous issued U.S. Patents in a manner that is consistent with its use here, including particularly U.S. Patent Nos. 6,696,423 (claims 1 and 7); 6,540,995 (claims 1, 5, 8, 11, 14, 16, 18, 21, and 23); and 6,482,405 (claim 1). As such, applicants respectfully submit that the phrase "at a site of the tumor or restenosis" adequately describes where the claimed compositions are to be administered.

Thus, given the nature of the claims considered in the context of the invention as a whole, applicants respectfully submit that the instant rejection regarding the phrase "at the site" has been addressed. Applicants further respectfully request that the rejection be withdrawn, and that the claims be allowed at this time.

VI.C. Rejection of Claim 63

Claim 63 has been rejected under 35 U.S.C. § 112, second paragraph, upon the contention that the phrase "the living animal body" lacks antecedent basis. Applicants have omitted the objected to phrase, and thus believe that the rejection has been addressed. Applicants respectfully request that the rejection be withdrawn.

VI.D. Rejection of Claims 27, 31, and 63

Claims 27, 31, and 63 have been rejected based on the contention that the phrase "living animal body, including a human, in need thereof" is indefinite. Applicants have amended claims 27 and 63 to delete the objected to phrase. These claims now recite *inter alia* methods of treating a subject having a tumor or restenosis by administering to the subject a composition at a site of the tumor or restenosis.

Accordingly, applicants respectfully submit that the instant rejection of claims 27, 31, and 63 has been addressed. Applicants further respectfully submit that as a result of the amendments and remarks presented hereinabove, claims 27, 31, and 63 are in condition for allowance. Applicants respectfully request that the instant rejection under 35 U.S.C. § 112, second paragraph, be withdrawn and the claims allowed at this time.

VI.E. Rejection of Claim 32

Claim 32 has been rejected upon several bases alleging that the claim is indefinite. Applicants respectfully traverse this rejection and point out that the claim recites administering a producer cell line at the site of the tumor or the restenosis. Thus, there is no basis for the Patent Office to assert that it is unclear if the site is *in vivo* or *in vitro*. There is also no basis to suggest that the claim must be limited to either alternative. As such, whether the claim encompasses a site that is *in vivo*, *in vitro*, or encompasses both, the Patent Office has articulated no reasonable rationale for requiring applicants to distinguish between these possibilities. Accordingly, applicants respectfully submit that the instant rejection is improper.

However, in an effort to facilitate the prosecution of the remaining claims, applicants have canceled claim 32. Thus, the instant rejection is believed to have been rendered moot.

VI.F. Rejection of Claim 63

Claim 63 has been rejected as indefinite based upon several contentions. Applicants have considered the rejection and these contentions, and traverse the rejection.

Initially, applicants respectfully submit that claim 63 recites *inter alia* a method for treating a tumor or restenosis by implanting a capsule having a core, wherein the core comprises the claimed packaging cells. Applicants submit that the capsules, if they are to encapsulate the producer cells, must have a core where the producer cells are located. Furthermore, the claim indicates that the packaging cells harbor (*i.e.* comprise) a retroviral vector carrying a DNA sequence encoding SDI-1 or a functional

fragment thereof and at least one DNA construct encoding the proteins required for the retroviral vector to be packaged. Additionally, the core is surrounded by a porous capsule wall that is permeable to the retroviral particles that are produced by the producer cells. Lastly, the capsule having a core is implanted into the living animal body, including a human, at a site of the tumor or restenosis. Applicants respectfully submit that when the disclosure of the instant application is considered in its entirety, the language of claim 63 clearly sets out the metes and bounds of the claim. Consequently, applicants respectfully submit that the instant rejection is improper, and request its withdrawal.

However, in an effort to facilitate the prosecution of the claim, applicants have amended claim 63 as follows. Claim 63 now recites a method for the treatment of a subject having a tumor or restenosis, the method comprising implanting into the subject at a site of the tumor a capsule comprising:

(a) a plurality of packaging cells comprising:
    (i) a retroviral vector comprising a DNA sequence encoding an SDI-1 polypeptide or a functional fragment thereof; and
    (ii) at least one DNA construct encoding a protein required for said retroviral vector to be packaged; and
(b) a porous capsule wall that is permeable to retroviral particles produced by the packaging cells.

Applicants respectfully submit that as a result of the amendments, the instant rejections of claim 63 have been addressed, and that claim 63 is now in condition for allowance. Accordingly, applicants request that the rejection of claim 63 under 35 U.S.C. § 112, second paragraph, be withdrawn and the claim allowed at this time.

VII. Claim Rejections under 35 U.S.C. § 103(a)

claims 13, 14, 19, 26, 27, 31, 32, 39, 40, 45, 48, 50, and 53 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Miller or Price in view of Nabel. These claims have also been rejected under this section over Gunzburg (PCT

International Patent Application WO 96/07748; hereinafter "Gunzburg") in view of Nabel. After careful consideration of the rejections and the Patent Office's bases therefor, applicants respectfully traverse the rejections and submit the following remarks.

VII.A. Obviousness Rejection over Miller or Price in view of Nabel

Claims 13, 14, 19, 26, 27, 31, 32, 39, 40, 45, 48, 50, and 53 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Miller or Price in view of Nabel. According to the Patent Office, Miller and Price taught stably transfected packaging cells producing retroviral particles. The Patent Office also asserts that the vector of Miller or Price has a 5' LTR having a U3-R-U5 structure and a 3' LTR having a completely or partially deleted U3 region replaced by a promoter followed by R and U5 as in claim 13. The Patent Office also asserts that the packaging cells are suspended in culture media, which is a "carrier". After careful consideration of the rejection and the Patent Office's bases therefor, applicants respectfully traverse the rejection and submit the following remarks.

Initially, applicants respectfully submit that culture medium is not a "carrier" as recited in claim 19. The Patent Office presents no scientific support for this contention, and applicants respectfully submit that there is no support for the contention that one of ordinary skill in the art would recognize culture medium as an "pharmaceutically acceptable carrier or diluent". Accordingly, the Patent Office has presented no evidence to support its contention that the element "pharmaceutically acceptable carrier or diluent" as recited in claim 19 is disclosed or suggested in any reference or is known by skilled artisans. As a result, applicants respectfully request that this assertion be withdrawn.

Continuing with the instant rejection, claim 13 recites the following: an isolated producer cell line stably transfected with a retroviral vector comprising in 5' to 3' order:

(a) a 5' LTR region of the structure U3-R-U5;

(b) a sequence encoding an SDI-1 polypeptide or a functional fragment thereof; and

(c) a 3' LTR region comprising a completely or partially deleted U3 region, wherein into said deleted U3 region has been cloned a polylinker sequence into which a regulatory element or a promoter has been inserted,

wherein said SDI-1 polypeptide or functional fragment thereof inhibits cell proliferation, and said isolated producer cell line comprises at least one DNA construct encoding a protein required for said retroviral vector to be packaged. Subsection (c) of claim 13 is also present in independent claims 39, 45, and 50. Thus, the claimed producer cell lines comprise a retroviral vector with a completely or partially deleted 3' U3 region, and also comprise a polylinker sequence into which a regulatory element or a promoter has been inserted.

Applicants respectfully submit that the Patent Office's assertion that the vector of Miller or Price is characterized by a 3' LTR having a completely or partially deleted U3 region replaced by a promoter followed by R and U5 is inaccurate. Looking at Miller first, the reference discloses several retroviral vectors, including N2, LNL6, LNSX, LNCX, and LXSN, the structures for which are disclosed in Figures 1 and 3 of the cited reference. Applicants respectfully submit that none of these vectors have 3' LTR deletions, and none of these vectors have promoters that have been inserted into a 3' LTR deletion. As is clearly shown in the reference, the vectors have complete, wild-type Moloney Murine Leukemia Virus (Mo-MuLV) 3' LTRs.

That the vectors of Miller have complete wild-type Mo-MuLV 3' LTRs is confirmed by review of the Genbank® database. Genbank® Accession No. J02255 is the complete genome of Mo-MuLV. Bases 7816-8332 correspond to the 3' LTR. Genbank® Accession No. SYNMMLPLN2 is Miller vector pLNSX. Genbank® Accession No. SYNMMLPLN3 is Miller vector pLNCX. Genbank® Accession No. SYNMMLPLN4 is Miller vector pLXSN. Careful consideration of the nucleotide sequences of each of these three vectors using the BLAST algorithm indicates that each and every one of these vectors has 100% identity with the 517 basepair 3' LTR of Mo-MuLV. These vectors are derivatives of Miller vectors N2 and LNL6, but the

derivation did not involve modification of the 3' LTR sequences, and thus N2 and LNL6 also have complete, unmodified 3' LTRs from Mo-MuLV. As a result, applicants respectfully submit that the Patent Office's characterization of the Miller vectors as having "a completely or partially deleted U3 region replaced by a promoter" is clearly erroneous. Thus, the combination of Miller and Nabel does not teach or suggest a retroviral vector with a "completely or partially deleted U3 region replaced by a promoter" as recited in claims 13, 39, 45, and 50.

Turning now to the vectors of Price, applicants respectfully submit that Price discloses the β-gal transducing vector called "BAG". The BAG vector was produced by cloning the *E. coli* β-galactosidase gene into the pDOL vector, which is derived from Mo-MuLV. Page 157 of Price states that "the wild-type Mo-MuLV LTR provided the promoter for the β-gal gene". Since the promoter of the wild-type Mo-MuLV is present in the 3' LTR U3 region, it is clear that the BAG vector has a wild-type 3' LTR. Thus, applicants respectfully submit that contrary to the Patent Office's contention, the BAG vector is not characterized by a completely or partially deleted U3 region replaced by a promoter as recited in claims 13, 39, 45, and 50. Accordingly, Price does not teach or suggest each and every element these claims.

Summarily, applicants respectfully submit that the combination of Miller or Price in view of Nabel does not support a *prima facie* case of obviousness under 35 U.S.C. § 103(a) because the cited combination does not teach or suggest each and every element of the claims. As discussed in more detail hereinabove, claims 13, 39, 45, and 50 recite producer cells lines comprising *inter alia* "a 3' LTR region comprising a completely or partially deleted U3 region, wherein into said deleted U3 region has been cloned a polylinker sequence into which a regulatory element or a promoter has been inserted". The combination of Miller and/or Price in view of Nabel does not disclose this element, and thus claims 13, 39, 45, and 50 are believed to be patentably distinguished from the asserted combinations. Claims 14, 19, 26, 27, 31, 32, 40, 48, and 53 all depend directly or indirectly from the distinguished claims, and thus are believed to be themselves distinguished from the cited combination. Claim 32 has

been canceled, and thus the rejection is believed to be moot as to this claim. Accordingly, applicants respectfully request that the rejection of claims 13, 14, 19, 26, 27, 31, 39, 40, 45, 48, 50, and 53 under 35 U.S.C. § 103(a) over Miller or Price in view of Nabel be withdrawn, and the claims allowed at this time.

VII.B. Obviousness Rejection Gunzburg in view of Nabel

Claims 13, 14, 19, 26, 27, 31, 32, 39, 40, 45, 48, 50, and 53 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Gunzburg in view of Nabel. According to the Patent Office, Gunzburg taught stably transfecting packaging cells producing retroviral particles having the structure described in claim 13. The Patent Office also asserts that the packaging cells were inherently suspended in culture medium, which is a "carrier" as in claim 19. The Patent Office contends that Gunzburg did not teach that the retroviral particles encoded SDI-1 or treating restenosis using retroviral particles encoding SDI-1. This defect is asserted to be cured by Nabel, which is asserted to teach the use of viral particles encoding SDI-1 into a patient to treat restenosis. From this, the Patent Office asserts that it would have been obvious to one of ordinary skill in the art at the time the instant invention was made to make a stably transfected packaging cell line that produces retroviral particles as taught by Gunzburg to make retroviral particles encoding SDI-1 as taught be Nabel. According to the Patent Office, one of ordinary skill in the art would have been motivated to use the retroviral particles of Gunzburg to deliver SDI-1 as taught by Nabel because the retroviral particles of Gunzburg had the advantage of being non-self-inactivating and had increased safety. After careful consideration of the rejection and the Patent Office's bases therefor, applicants respectfully traverse the rejection and submit the following remarks.

Initially, applicants respectfully submit that culture medium is not a "carrier" as recited in claim 19. The Patent Office's attention is directed to the discussion presented above regarding this contention. Given that this contention is unsupported by any scientific evidence in the record, applicants respectfully request that this contention be withdrawn.

Continuing with the instant rejection, applicants respectfully submit that the combination of Nabel and Gunzburg does not support a rejection under § 103(a) because even assuming *arguendo* that the Patent Office's remaining assertions related to the combination are accurate, the Gunzburg reference is not prior art as to the instant application. Applicants respectfully submit that Gunzburg was published on March 14, 1996. The instant application claims priority to DK 1157/95, which was filed October 13, 1995. The priority date of the instant application is prior to the publication date of Gunzburg. Furthermore, applicants respectfully submit that DK 1157/95, a true and accurate copy of the English translation of which is attached hereto as **Exhibit A**, discloses ProCon vectors (*see* particularly page 3 and Figure 1 of **Exhibit A**). Furthermore, DK 1157/95 discloses DK 1017/94, the patent application filed in Denmark to which WO 96/07748 itself claims priority (*see* page 3 of DK 1157/95). Applicants respectfully submit, therefore, that the reference to WO 96/07748 in the instant application is merely duplicative. Accordingly, applicants respectfully submit that they are fully entitled to the October 13, 1995 priority date of DK 1157/95. As a result, applicants respectfully submit that Gunzburg does not qualify as "prior art" with respect to the instant application.

Thus, applicants respectfully submit that a *prima facie* case of obviousness of claims 13, 14, 19, 26, 27, 31, 32, 39, 40, 45, 48, 50, and 53 over Gunzburg in view of Nabel has not been presented. Claim 32 has been canceled, and thus the rejection is believed to be moot as to this claim. Accordingly, applicants respectfully request that the rejection of claims 13, 14, 19, 26, 27, 31, 39, 40, 45, 48, 50, and 53 over Gunzburg in view of Nabel be withdrawn, and the claims allowed at this time.

Conclusions

In light of the above amendments and remarks, applicants respectfully submit that claims 1-4, 9-11, 13-16, 19-21, 23, 26, 27, 31, 32, 39-43, 45-48, 50-53, 58-59, 61, and 63 are in condition for allowance at this time, and respectfully solicit a Notice of Allowance to that effect.

If any small matter should remain outstanding after the Patent Examiner has had an opportunity to review the above Remarks, the Patent Examiner is respectfully requested to telephone the undersigned patent attorney in order to resolve these matters and avoid the issuance of another Official Action.

DEPOSIT ACCOUNT

The Commissioner is hereby authorized to charge any fees associated with the filing of this correspondence to Deposit Account No. **50-0426**.

Respectfully submitted,

JENKINS, WILSON & TAYLOR, P.A.

Date: 09/15/2004 By: _____

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Exhibit A

Denmark Patent Application Serial No.

DK 1157/95

filed October 13, 1995

(provided as a true and accurate copy of the English translation)

KN 9

Kongeriget Danmark

Patent application No.: 1157/95

Date of filing: 13 Oct 1995

Applicants: GSF - Forschungszentrum fuer Umwelt und Gesundheit GmbH, Ingolstaedter Landstr. 1, Neuherberg, D-85758 Oberschleissheim, DE; Bavarian Nordic Research Institute A/S, Smedeland 26B, DK-2600 Glostrup, DK

This is to certify the correctness of the following information:

The attached photocopy is a true copy of the following document:

- The specification, claims, abstract and drawings as filed with the application on the filing date indicated above.

Erhvervsministeriet
Patentdirektoratet

TAASTRUP 12 Aug 1996

Jytte Hansen

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Kontorfuldmægtig

BN 9 DK

13 Oktober 1995

Titel: Retrovirale vectorer og deres anvendelse.

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Viral vectors carrying SDI-1 or antisense SDI-1 genes under transcriptional control of target cell specific regulatory sequences or X-ray inducible promoters, and their use.

The present invention relates to targeted expression of SDI-1 or antisense SDI-1 genes, and especially the use of the rodent WAP (Whey Acidic Protein) and the MMTV (Mouse Mammary Tumor Virus) regulatory sequences for targeted expression of linked SDI-1 or antisense SDI-1 genes in human mammary carcinoma cells.

Background of the Invention

Mammary carcinoma is the most frequent tumour in women (Miller and Bulbrook, 1986). Up to now the conventional therapy involves surgical removal of the primary tumour followed by a chemo- or radiation therapy. Depending on the tumour stage, the rate of relapse is quite high and has a fatal outcome in most cases. A major problem is the elimination of all metastases and micrometastases. Both this, as well as the serious side effects for the patient caused by conventional treatment, favour the development of a gene therapy approach (for a review on gene therapy see Anderson, 1992). One such approach could involve the use of a modified retrovirus or retroviral vector, to specifically deliver therapeutic genes to mammary carcinoma cells. The therapeutic gene would either inhibit the proliferation of tumour cells or kill the tumour cells after infection (suicide or toxin genes). The great advantage of a viral system would be that the virus particles can be spread in the blood stream similarly to metastazising tumour cells, which will make it possible to eliminate micrometastases long before they can be detected by conventional methods. Systemic delivery however poses the problem of the ability to target the therapeutic retroviral vector's expression only to the tumour cells.Therefore a control element is required to ensure that the transferred retroviral vector is only active in tumour cells.

Retroviral vector systems consist of two components:

1) the retroviral vector itself is a modified retrovirus (vector plasmid) in which the genes encoding for the viral proteins have been replaced by therapeutic genes and/or marker genes to be transferred to the target cell. Since

the replacement of the genes encoding for the viral proteins effectively cripples the virus it must be rescued by the second component in the system which provides the missing viral proteins to the modified retrovirus.

The second component is:

2) a cell line that produces large quantities of the viral proteins, however lacks the ability to produce replication competent virus. This cell line is known as the packaging cell line and consists of a cell line transfected with one or more plasmids carrying the genes enabling the modified retroviral vector to be packaged.

To generate the packaged vector, the vector plasmid is transfected into the packaging cell line. Under these conditions the modified retroviral genome including the inserted therapeutic and marker genes is transcribed from the vector plasmid and packaged into the modified retroviral particles (recombinant viral particles). This recombinant virus is then used to infect target cell's in which the vector genome and any carried marker or therapeutic genes becomes integrated into the target cell's DNA. A cell infected with such a recombinant viral particle cannot produce new vector virus since no viral proteins are present in these cells. However the DNA of the vector carrying the therapeutic and marker genes is integrated in the cell's DNA and can now be expressed in the infected cell.

A major consideration when considering the use of systemic retroviral delivery gene therapy, both from a safety stand point and from a purely practical stand point is, as mentioned above the targeting of retroviral vectors. It is clear that therapeutic genes carried by vectors should not be indiscriminately expressed in all tissues and cells, but rather only in the requisite target cell. This is especially important if the genes to be transferred are toxin genes aimed at ablating specific tumour cells. Ablation of other, nontarget cells would obviously be very undesirable.

A number of retroviral vector systems have been previously described that should allow targeting of the carried therapeutic genes (reviewed in Salmons and Gunzburg, 1993). Most of these approaches involve either limiting the infection event to predefined cell types or using heterologous promoters to direct expression of linked heterologous therapeutic or marker genes to specific cell

types. Heterologous promoters are used which should drive expression of linked genes only in the cell type in which this promoter is normally active.
In danish patent application no. 1017/94 the principle and construction of a new type of retroviral vector, the ProCon-vector, carrying various types of tissue specific regulatory elements are described:

The retroviral genome consists of an RNA molecule with the structure R-U5-gag-pol-env-U3-R. During the process of reverse transcription, the U5 region is duplicated and placed at the right hand end of the generated DNA molecule, whilst the U3 region is duplicated and placed at the left hand end of the generated DNA molecule. The resulting structure U3-R-U5 is called LTR (Long Terminal Repeat) and is thus identical and repeated at both ends of the DNA structure or provirus (Varmus, 1988). The U3 region at the left hand end of the provirus harbours the promoter and transcriptional regulatory sequences (see below). This promoter drives the synthesis of an RNA transcript initiating at the boundary between the left hand U3 and R regions and terminating at the boundary between the right hand R and U5 region. This RNA is packaged into retroviral particles and transported into the target cell to be infected. In the target cell the RNA genome is again reverse transcribed as described above.

In the ProCon-vector the right-hand U3 region is altered (Fig. 1), but the normal left-hand U3 structure is maintained (Fig. 1); the vector can be normally transcribed into RNA utilizing the normal retroviral promoter located within the left-hand U3 region (Fig. 1). However the generated RNA will only contain the altered right-hand U3 structure. In the infected target cell, after reverse transcription, this altered U3 structure will be placed at both ends of the retroviral structure (Fig. 1).

If the altered region carries a polylinker (see below) instead of the U3 region then any promoter, including those directing tissue specific expression such as the WAP promoter (see below) can be easily inserted. This promoter can then be utilized exclusively in the target cell for expression of linked genes carried by the retroviral vector. Additionally DNA segments homologous to one or more cellular sequences can be inserted into the polylinker for the purposes of gene targeting, by homologous recombination. Other means of directing gene expression to target tissue is to use X-ray inducible promoters.

The expression vectors used for the purpose of the invention need not be of the ProCon type, but can be any conventional vector carrying heterologous DNA sequence(s) under transcriptional control of the WAP or MMTV regulatory sequences. The vector used for the purpose of the invention can be a retroviral vector of conventional type i.e. with the WAP or MMTV promoters used as internal promoters, i.e. LTR-neo-*WAP-therapeutic-gene*-LTR, but is most preferably a retroviral vector of the ProCon type.

Vector constructs carrying various types of mammary gland specific regulatory elements have been tested in mice where expression of a marker gene driven by the regulatory elements in the hormonally stimulated mammary gland could be achieved (DK patent application no. 1017/94). One regulatory element demonstrated to give rise to expression in the pregnant and lactating mouse mammary gland is a small region of the rodent WAP promoter (Kolb et al., 1994). This gene is only expressed in the pregnant and lactating mammary glands of rodents and has no human homologue (Hennighausen, 1992). It is therefore not predictable that this regulatory element will function at all to direct expression in human cells and/or allow expression in human mammary carcinoma cells.

It was thus quite unexpected when the inventors of the present invention found that a 578bp element of the WAP promoter is able to direct expression of a linked marker gene (β-gal) in primary human mammary carcinoma cells.

The therapeutic gene to be delivered to the tumor cells is another important element in the construction of viral vectors for use in cancer therapy, and here the "Senescent Derived Inhibitor" SDI-1 and the corresponding SDI-1 gene and antisense SDI-1 genes are of particular interest:

Dividing cells undergo a cyclical programme that culminates in cell division. A normal event in this cell cycle programme is the replication of the cellular DNA. Just prior to this DNA synthesis, there is a pause to allow proof reading of the DNA, ensuring that any damage or mutations are repaired and not passed on to daughter cells. This checkpoint is regulated by programmed gene expression. A second, similar checkpoint occurs later on after DNA synthesis, just before the cell divides into two new cells, presumable for the same purpose. Senescent or aged cells are permanently arrested at one of these checkpoints. Recently, Olivia Pereira-Smith, Jim

Smith and colleagues have identified three cDNAs that cause growth arrest when transfected into young, actively dividing cells (Noda et al., 1994). One of these sequences, SDI-1 has also been independently cloned by other groups as a cyclin-dependent kinase inhibitor (CIP1; Harper et al., 1993), a gene that is induced by p53 (WAF1; El-Diery et al., 1993) and a gene involved in melanocyte differentiation (MDA6; Jiang and Fisher, 1993). Thus the same gene has a central role in cellular processes that have in common the loss of cell proliferation which implicates this gene as being involved in cell cycle control. SDI-1 has been shown to be overexpressed in senescent cells, quiescent cells or cultured primary cells undergoing crisis (Noda et al., 1994; Rubelji et al., 1994), suggesting a role in the maintenance of DNA synthesis inhibition (Johnson et al., 1994). Further, evidence has been presented suggesting that the SDI-1 mediated inhibition of DNA synthesis occurs via an inhibition of Cdk activity (Nakanishi et al., 1995). These findings, together with the demonstration that SDI-1 can inhibit cell growth of young dividing cells, demonstrates that this gene will be useful for gene therapy to inhibit the growth of rapidly proliferating cells in diseases such as restenosis, in which smooth muscle cells inappropriately divide, or various cancers.

To achieve this, a cDNA encoding SDI-1 would be placed under the control of a promoter in an expression cassette and delivered by any standard gene transfer technique, preferably in a retroviral vector. Rapidly dividing cells would then be infected with the vector. A retroviral vector based upon murine leukemia virus would offer the advantage that they are ony able to successfully deliver genes to dividing cells, thereby avoiding that surrounding nondividing cells are infected. The infected cells would then express SDI-1 and become arrested in the cell cycle, preventing further cell divisions and possibly inducing senescence.

WO patent application No. 95/06415 describes the sequence of the SDI-1 gene and numerous therapeutical uses of the gene and the encoded protein, as well as antisense nucleotides capable of inhibiting the expression of the SDI-1 gene. The potential use of the SDI-1 gene and the encoded protein in cancer treatment is described in on pages 48-57 of the specification. Here it is suggested that the SDI-1 gene or protein is used in combination with conventional chemotherapy:

The premise of chemotherapy is that cancer cells grow more rapidly than normal cells, and hence are more sensitive to cytotoxic agents than normal cells. Many chemotherapeutic agents exert their effect during a specific phase or set of phases of the cell cycle. And because only a fraction of tumor cells are in a specific phase at any given time, such drugs must generally be provided in repeated administration. The use of the SDI gene or protein to synchronize or maximize the percentage of cells that are in a particular phase of the cell cycle at the time of administering the chemotherapeutic, provides a means to increase the effectivity of chemotherapy.

For the delivery of the SDI-1 gene or antisense gene to cells it is suggested to use viral or retroviral vectors and to use tissue specific promoters in order to confine the therapeutic effect to a desired site or tissue (pages 59 and 67).

WO-A1-95/06415 do not however disclose the use of the mammary gland specific WAP and MMTV regulatory sequences for the expression of the SDI-1 gene and antisense SDI-1 genes.

Summary of the Invention

The invention then, inter alia, comprises the following, alone or in combination:

A replication-defective retroviral vector carrying a SDI-1 gene or an antisense SDI-1 gene under transcriptional control of target cell specific regulatory elements or promoters or X-ray inducible promoters;

a replication-defective retroviral vector as above, wherein the vector comprises a 5' LTR region of the structure U3-R-U5; one or more sequences selected from coding and non-coding sequences; and a 3' LTR region comprising a completely or partially deleted U3 region wherein said deleted U3 region is replaced by a polylinker sequence containing the target cell specific regulatory elements or promoters or an X-ray inducible promoter, followed by the U5 and R region,

characterized in that at least one of the coding sequences is a SDI-1 gene or an antisense SDI-1 gene;

a replication-defective retroviral vector as any above, wherein the target cell specific regulatory element is the WAP or MMTV regulatory sequences;

a replication-defective retroviral vector as above, wherein the regulatory sequence is the 578bp element of the WAP promoter-HGH gene hybrid or any other element/region of the WAP regulatory sequence conferring mammary specific expression;

a replication-defective retroviral vectoras above, wherein the regulatory sequence is the U3 region of MMTV or subregions thereof conferring mammary specific expression;

a packaging cell line preferably of rodent, canine, feline or human origin or a packaging cell line histocompatible with human tissue harbouring:

1) a retroviral vector as any above

2) at least one retroviral or recombinant retroviral construct coding for proteins required for said retroviral vector to be packaged;

a recombinant vector virus particle obtained by culturing the packaging cell line as above under suitable conditions optionally followed by isolation of the recombinant vector virus produced;

a pharmaceutical composition comprising the recombinant vector virus particle as above or a packaging cell line as above;

a method for the treatment of breast cancer comprising administering to a human in need thereof a recombinant vector virus particle as above or a packaging cell line as above;

a method for the treatment of restenosis comprising administering to a human in need thereof a recombinant vector virus particle as above or a packaging cell lineas above;

a retroviral provirus integrated in the human genome carrying a DNA-construct comprising a SDI-1 gene or an antisense SDI-1 gene under transcriptional control of the WAP or MMTV regulatory sequences or an X-ray inducible promoter; and

a human cell, containing a DNA construct carrying a SDI-1 gene or an antisense SDI-1 gene under transcriptional control of the WAP or MMTV regulatory sequences or an X-ray inducible promoter.

The WAPgal and the MMTVgal constructs are of particular interest for the purposes of the present invention because the regulatory elements conferring tissue specificity are both derived from the rodent system. This may become an important safety feature because the use of human regulatory sequences in a retroviral vector could cause problems because homologous recombinations between the vector carried sequences and the corresponding cellular may cause genome instability.

The retroviral vector is based preferably either on a BAG vector (Price *et al.*, 1987) or an LXSN vector (Miller and Rosman, 1989), but may be based on any other retroviral vector.

The retroviral vector preferably comprises a coding sequence selected from one or more elements of the group consisting of marker genes, and therapeutic genes.

Said marker genes are preferably selected from the group consisting of marker genes which codes for proteins such as ß-galactosidase, neomycin, alcohol dehydrogenase, puromycin, hypoxanthine phosphoribosyl transferase (HPRT), hygromycin and secreted alkaline phosphatase

Another embodiment of the invention envisages the alteration or partial deletion of at least one retroviral sequence required for the integration of the retrovirus.

The term "SDI-1 gene" means any nucleotide sequence coding for the SDI-1 protein, e.g SDI-1 cDNA, or any derivative thereof capable of inducing cellular quiescense, including nucleotide sequences that codes for only a fraction of the SDI-1 protein, e.g. amino acid residues 1-70, 5-70, 10-70, 15-70, 20-70, 25-70, 30-70, 35-70, or 40-70.

The term "antisense SDI-1 gene" means any nucleotide capable of directing the synthesis of an RNA that can inhibit the expression of SDI-1 by formation of a triplex structure.

The packaging cell line is preferably selected from an element of the group consisting of Ψ-2, Ψ -Crypt, Ψ-AM, GP+E-86, PA317 and GP+envAM-12, or of any of these transfected with recombinant constructs allowing expression of surface proteins from other enveloped viruses.

A further embodiment of the invention provides therapeutical method for introducing the SDI-1 gene or antisense SDI-1 genes into human cells *in vitro* and *in vivo* comprising transfecting a packaging cell line of a retroviral vector system with a retroviral vector carrrying one of these heterologous DNA sequences under transcriptional control of the WAP or MMTV regulatory sequences and infecting a target cell population with recombinant retroviruses produced by the packaging cell line.

According to the invention the term "polylinker" is used for a short stretch of artificially synthesized DNA which carries a number of unique restriction sites allowing the easy insertion of any promoter or DNA segment. The term "heterologous" is used for any combination of DNA sequences that is not normally found intimately associated in nature.

The following example will illustrate the invention further. The example is however in no way intended to limit the scope of the present invention as obvious modifications will be apparent, and still other modifications and substitutions will be apparent to anyone skilled in the art.

The recombinant DNA methods employed in practicing the present invention are standard procedures, well known to those skilled in the art, and described in detail, for example, in "Molecular Cloning" (Sambrook et al. 1989) and in "A Practical Guide to Molecular Cloning" (Perbal, 1984).

Example 1

Mammary gland specific expression after infection with ProCon Vectors carrying mammary specific promoters.

In the murine leukemia virus (MLV) retroviral vector known as BAG (Price et al., 1987) the ß-galactosidase gene is driven by the promiscuous (i.e. non-tissue specific) MLV promoter in the U3 region of the LTR (Fig. 1). According to the present invention a derivative of the BAG vector has been constructed in which the MLV promoter (U3) located within the 3'LTR (Fig. 1) has been deleted by PCR. At this position a polylinker was inserted containing the restriction sites *Sac*II and *Mlu*I allowing the facile introduction of heterologous promoters. The BAG vector lacking the U3 is expressed from the MLV promoter (U3) within the 5'LTR when introduced into a packaging cell line. As a result of the usual rearrangements occurring in the retroviral genome during its life cycle, following infection of its target cell, the polylinker will be duplicated at both ends of the retroviral genome as described in danish patent application no. 1017/94. Thereby a retroviral vector can be constructed in which the expression of the ß-galactosidase gene of BAG in the target cell will be controlled by any heterologous promoter inserted into the polylinker (Fig. 1).

According to the principle set forth above the following specific promoters have been inserted into the polylinker region or the modified BAG vector:

The Mouse Mammary Tumour Virus (MMTV) U3-Region (mtv-2) without the inverted repeats, which contains the MMTV promoter as well as a region that confers responsiveness to glucocorticoid hormones and a region containing an element that directs expression to the mammary gland.

The Whey Acidic Protein (WAP) promoter - Human Growth Hormone hybrid (Kolb et al., 1994) encompassing the positions -447 to +131 (with the transcription initiation site defined as +1), contains an element which controls the

expression of WAP so that it is only produced in the mammary glands of pregnant and lactating rodents.

The control of the ß-galactosidase gene expression by promoters inserted into the polylinker has been validated by infection studies using the constructed MMTV and WAP retroviral vectors to infect various cells.

To produce retroviral vector particles, the MMTV and WAP ProCon vectors have been transfected into the packaging cell line GP+E86 (Markowitz et al., 1988) or PA 317 (Miller and Buttimore, 1986) . After selection for neomycin resistance, which is encoded by the vector, stable populations and clones of recombinant ProCon virus producing cells were obtained. These clones and populations were producing recombinant virus into the cell culture medium. Alternatively 2 to 3 days after transfection (without selection) cell culture supernatant containing recombinant virus was harvested. Virus containing supernatant was used to infect explanted normal primary human mammary tissue obtained from reduction mammaplasties. Since it is known that these promoters are responsive to pregnancy hormones, the tissue was cultivated in the presence of such hormones. The expression of the marker gene was determined by a quantitative ß-gal assay which is based on the detection of ß-galactosidase activity by chemiluminescence. In all the experiments the original, non-tissue specific BAG-vector was used as a positive control. All of the analysed samples showed ß-galactosidase expression (Fig. 2) in three independent experiments. It has thus been demonstrated for the first time that the WAP regulatory elements as well as the MMTV-U3 region can drive the expression of a gene within a MLV retroviral vector in primary human mammary gland cells.

To determine whether these regulatory sequences are active in human mammary tumours as well, primary explants of human mammary tumours were infected with WAPgal. A few days later the tumour organoids were analysed for ß-gal expression as in the experiments described above. In this experiment the human mammary tumour cells infected with the WAPgal retroviral vector showed ß-gal expression (Fig. 3a). In another experiment it was demonstrated that the MMTVgal (125.gal) and the non-tissue specific BAG construct also express the ß-

gal gene in primary normal human mammary cells(i.e. non tumor derived cells) (Fig. 3b).

Example 2

Expression of the SDI-1 cDNA has has been tested using SDI-1 delivered by a retroviral vector and expressed under the transcriptional control of the inducible MMTV promoter. The retroviral vector used is of the ProCon type and carries the SDI-1 cDNA downstream of the 5'LTR and the MMTV U3 region inserted into the polylinker in the 3'LTR (Fig. 4). The vector also carries a neomycin (G418) resistance gene under the transcriptional control of the SV40 promoter (Fig. 5). The vector pLXS-SDI-1, was constructed using SDI-1 cDNA generated by polymerase chain reaction on the plasmid pSDI-1 (Noda et al., 1994) using two primers positioned at the 5' and 3' ends of the cDNA carrying heterologous 5' extensions. The sequence of the left hand PCR primer is 5' TATGGACGTC -TCCCTGCCGAAGTCAGTT 3' and the sequence of the right hand primer is 5' TATGGGATCC - GGCAGAAGATGTA GAGCG 3' . The 5' extentions harbor the sequence for an *Aat* II or a *Bam* HI restriction site on the left hand and right hand primers respectively. After digestion of the generated PCR product with *Aat* II and *Bam* HI , the SDI-1 cDNA was inserted into the *Aat* II and *Bam* HI sites of the Procon vector p125 carrying the MMTV promoter to produce the plasmid p125.SDI, whereafter the 4.9 kb *Afl* II - *Aat* II fragment of this plasmid was ligated to the *Afl* III-*Eco* RI fragment of pLXSN after blunt ending of both fragments. After introduction of the vector into the packaging cell line PA 317, the vector virus produced was used to infect cells, in this case the human bladder carcinoma derived EJ cell line (Paranda et al., 1982). G418 resistant cell clones have been isolated and analysed for acquisition and expression of SDI-1 and for their growth properties. Because of the use of a ProCon vector, after the infection event, the SDI-1 is placed under the transcriptional control of the glucocorticoid inducible MMTV promoter. Clones that have acquired the SDI-1 gene show a reduced growth rate when SDI-1 gene expression is induced from the MMTV promoter by treatment of the cells with the synthetic glucocorticoid hormone, dexamethasone.

The ability of dexamethasone to turn on SDI-1 expression and the resulting growth inhibition has been examined in various ways:

Figure 6 shows the result of S1 analysis of expression from LXS-125 SDI-1 infected EJ cells; only infected cells synthesise the expected transcript detected as a 79 nucleotide (nt) fragment after treatment with dexamethasone, On longer exposures transcripts can also be detected from the same cells grown in the absense of dexamethasone. Roughly equal amounts of RNA were analysed as can be seen in lanes 5-8 examining the expression of a cell encoded gene GAPDH.

Figure 7 shows the result of fluoroscent activated cell sorting of cells to determine the proportion of cells in the various stages of the cell cycle. EJ cells infected with the LXS-125 SDI-1 were grown in the presence of dexamethasone (+DEX) or in the absence of dexamethasone (-DEX). The cells were stained for DNA content and analyzed on a cell sorter. The percentage of cells in the various stages of the cell cycle is given on the figure. The treatment with dexamethasone results in a greater proportion of cells in G_0/G_1 phase and correspondingly less cells in the S phase.

Figure 8 shows the inhibition of cell growth measured by Giemsa staining of cells. EJ cell clones infected with LXS-125 SDI (EJ LXS-SDI) and noninfected cells were seeded in multiwell plates (5.000 cells per well) and allowed to grow for 5 days either in the presence (+D) or in the absence (-D) of dexamethasone . The infected EJ cells but not the non-infected EJ cells grow slower in the presence of dexamethasone suggesting that the expression of SDI (and not a nonspecific action of the dexamethasone) is responsible for the reduced growth rate of these cells.

An inducible promoter would not necessarily have to be used in SDI-1 carrying retroviral vectors for eventual gene therapy, though it may be useful in some instances.

Example 3

A second therapeutic use of SDI-1 involves the expression of an antisense SDI-1 to reduce the expression of endogenous SDI-1. This will prevent cells pausing to check DNA integrity and repair of the DNA before new DNA synthesis begins in preparation for the next cell division. If cells expressing antisense SDI-1 are treated with DNA damaging agents such as mutagens, carcinogens or irradiation (e.g. gamma, U.V.), the efficiency of DNA damage repair will be severely reduced because the cell will not pause to permit proof reading and repair. These cells will accumulate so much DNA damage that they are no longer viable. Again, it may be useful to be able to control the expression of the gene with inducible promoters.

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Claims:

1. A replication-defective retroviral vector carrying a SDI-1 gene or an antisense SDI-1 gene under transcriptional control of target cell specific regulatory elements or promoters or X-ray inducible promoters.

2. A replication-defective retroviral vector according to claim 1, wherein the vector comprises a 5' LTR region of the structure U3-R-U5; one or more sequences selected from coding and non-coding sequences; and a 3' LTR region comprising a completely or partially deleted U3 region wherein said deleted U3 region is replaced by a polylinker sequence containing the target cell specific regulatory elements or promoters or an X-ray inducible promoter, followed by the U5 and R region, characterized in that at least one of the coding sequences is a SDI-1 gene or an antisense SDI-1 gene.

3. A replication-defective retroviral vector according to claim 1-2, wherein the target cell specific regulatory element is the WAP or MMTV regulatory sequences.

4. A replication-defective retroviral vector according to claim 3, wherein the regulatory sequence is the 578bp element of the WAP promoter-HGH gene hybrid or any other element/region of the WAP regulatory sequence conferring mammary specific expression.

5. A replication-defective retroviral vector according to claim 3, wherein the regulatory sequence is the U3 region of MMTV or subregions thereof conferring mammary specific expression.

6. A packaging cell line preferably of rodent, canine, feline or human origin or a packaging cell line histocompatible with human tissue harbouring:

1) a retroviral vector according to claims 1-5

2) at least one retroviral or recombinant retroviral construct coding for

proteins required for said retroviral vector to be packaged.

7. A recombinant vector virus particle obtained by culturing the packaging cell line according to claim 6 under suitable conditions optionally followed by isolation of the recombinant vector virus produced.

8. A pharmaceutical composition comprising the recombinant vector virus particle according to claim 7 or a packaging cell line according to claim 6.

9. A method for the treatment of breast cancer comprising administering to a human in need thereof a recombinant vector virus particle according to 7 or a packaging cell line according to claim 6.

10. A method for the treatment of restenosis comprising administering to a human in need thereof a recombinant vector virus particle according to claim 7 or a packaging cell line according to claim 6.

11. A retroviral provirus integrated in the human genome carrying a DNA-construct comprising a SDI-1 gene or an antisense SDI-1 gene under transcriptional control of the WAP or MMTV regulatory sequences or an X-ray inducible promoter .

12. A human cell, containing a DNA construct carrying a SDI-1 gene or an antisense SDI-1 gene under transcriptional control of the WAP or MMTV regulatory sequences or an X-ray inducible promoter.

Abstract

The present invention relates to a replication-defective retroviral vector carrying a SDI-1 gene or an antisense SDI-1 gene under transcriptional control of target cell specific regulatory elements or promoters.

Construction of a U3 minus BAG-vector (MLV)

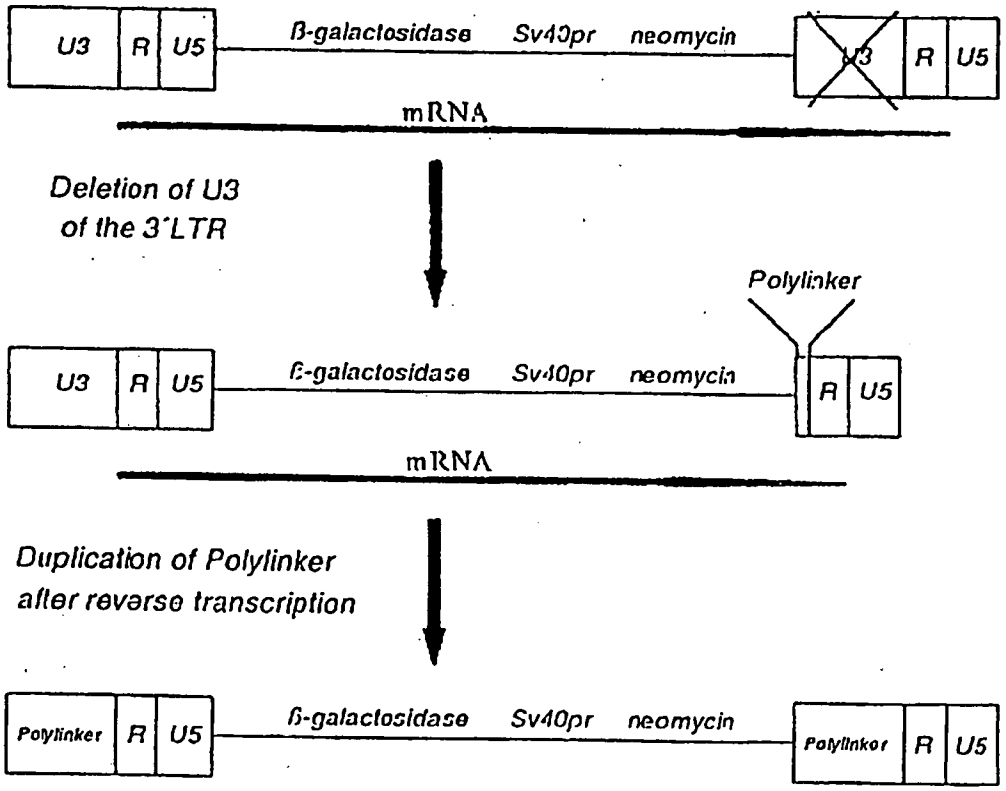


FIG 1

ß-Gal Expression of vector contructs after infection of primary hum. mgl cells

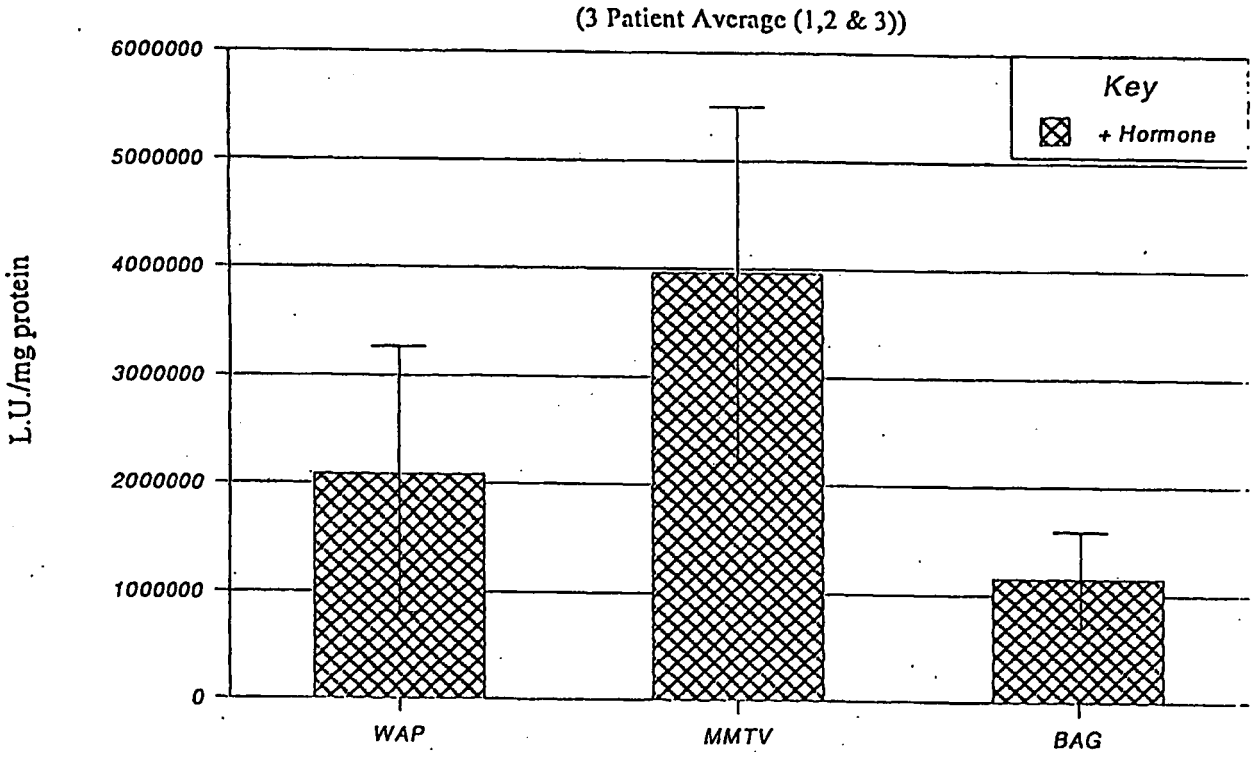


FIG 2

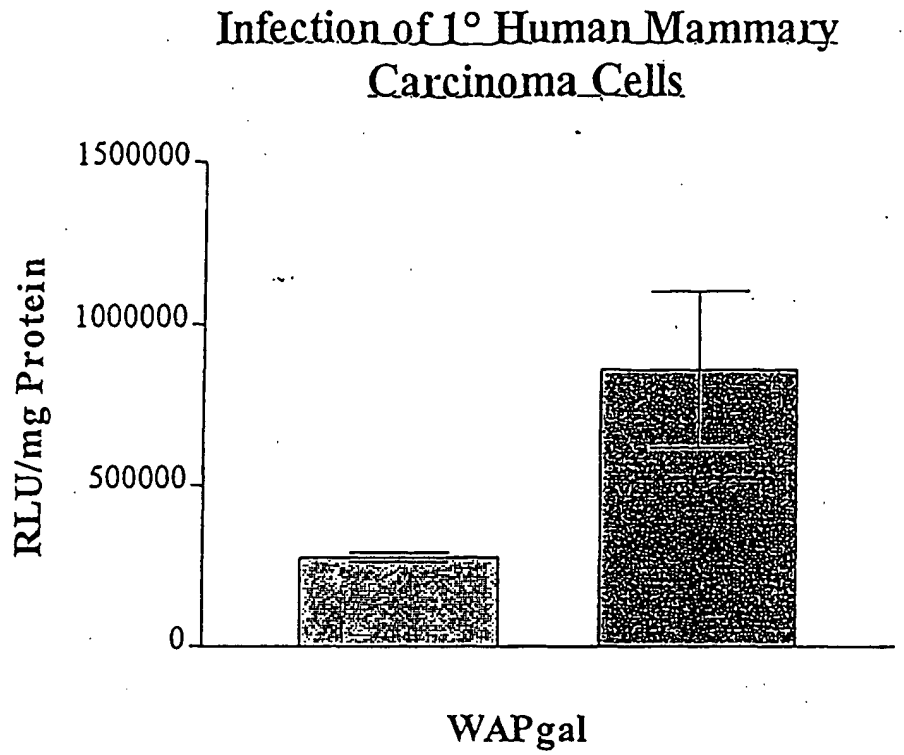

Infection of 1° Human Mammary Carcinoma Cells
1500000
1000000
500000
0
RLU/mg Protein
WAPgal

FIG 3.a

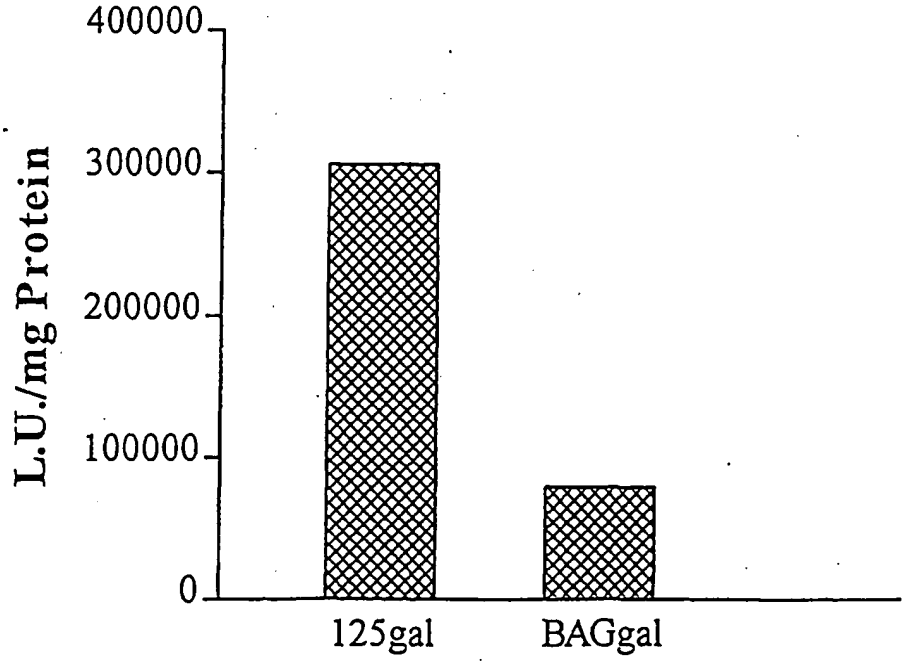

400000
300000
200000
100000
0
L.U./mg Protein
125gal
BAGgal

FIG 3.b

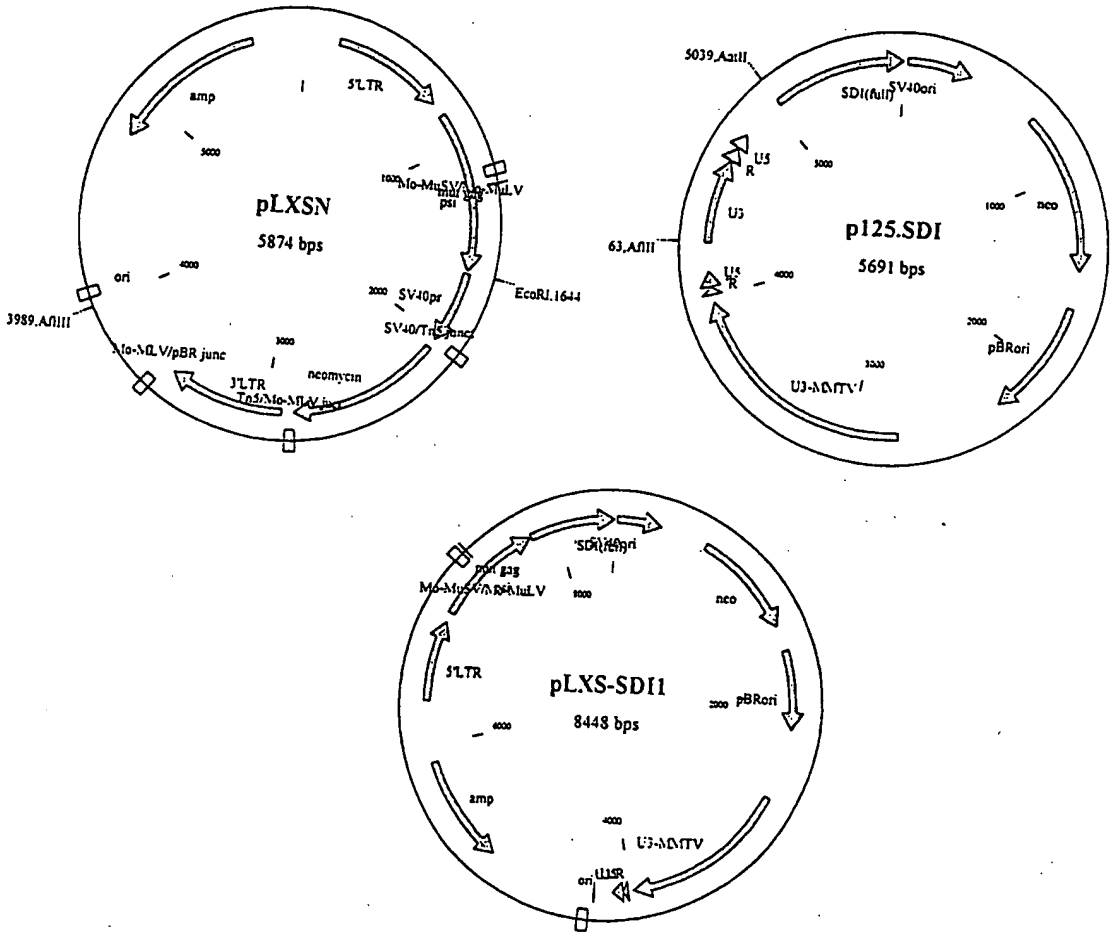

pLXSN
5874 bps
amp
5'LTR
ori
3989.AflII
EcoRI.1644
SV40pr
neomycin
3'LTR
p125.SDI
5691 bps
5039.AatII
SDI(full)
SV40ori
U5
R
U3
63.AflII
pBRori
pLXS-SDI1
8448 bps
5'LTR
amp
pBRori
neo
ori

FIG 4

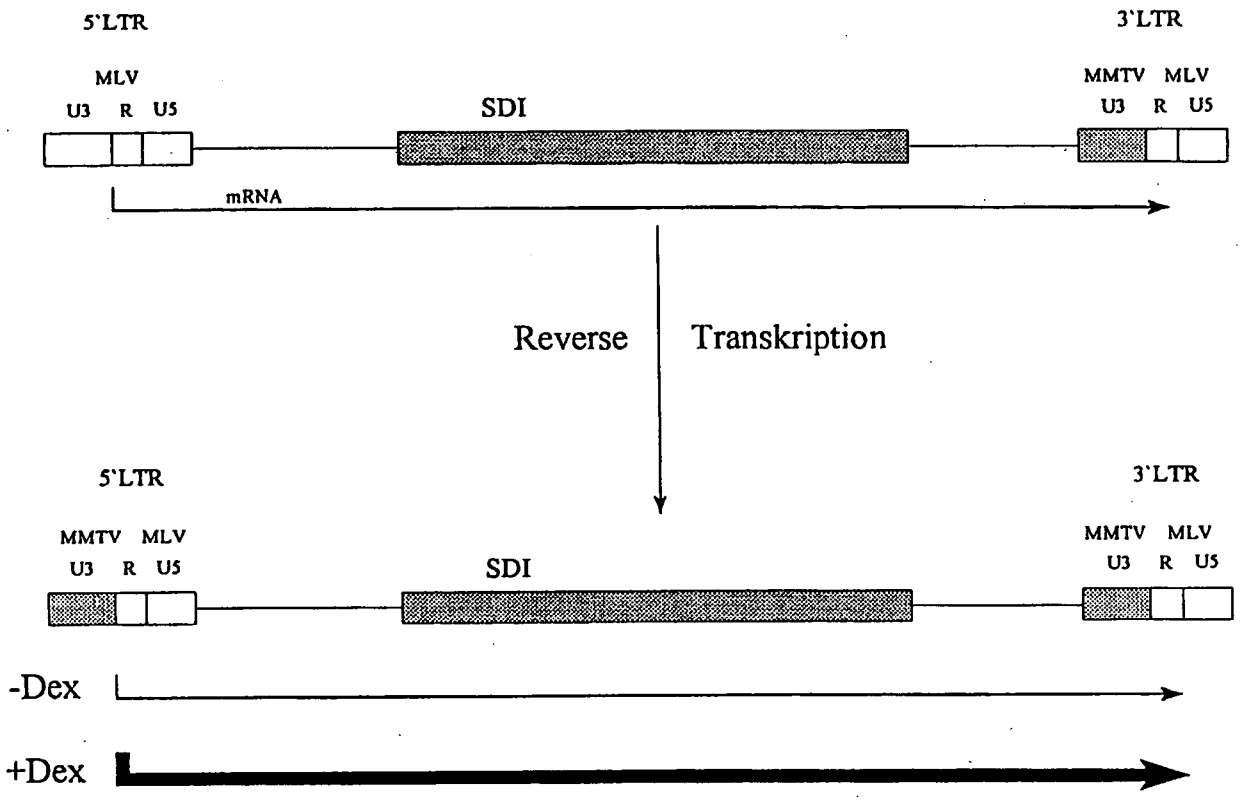

5'LTR
MLV
U3 R U5
SDI
3'LTR
MMTV MLV
U3 R U5
mRNA
Reverse Transkription
5'LTR
MMTV MLV
U3 R U5
SDI
3'LTR
MMTV MLV
U3 R U5
-Dex
+Dex

FIG 5

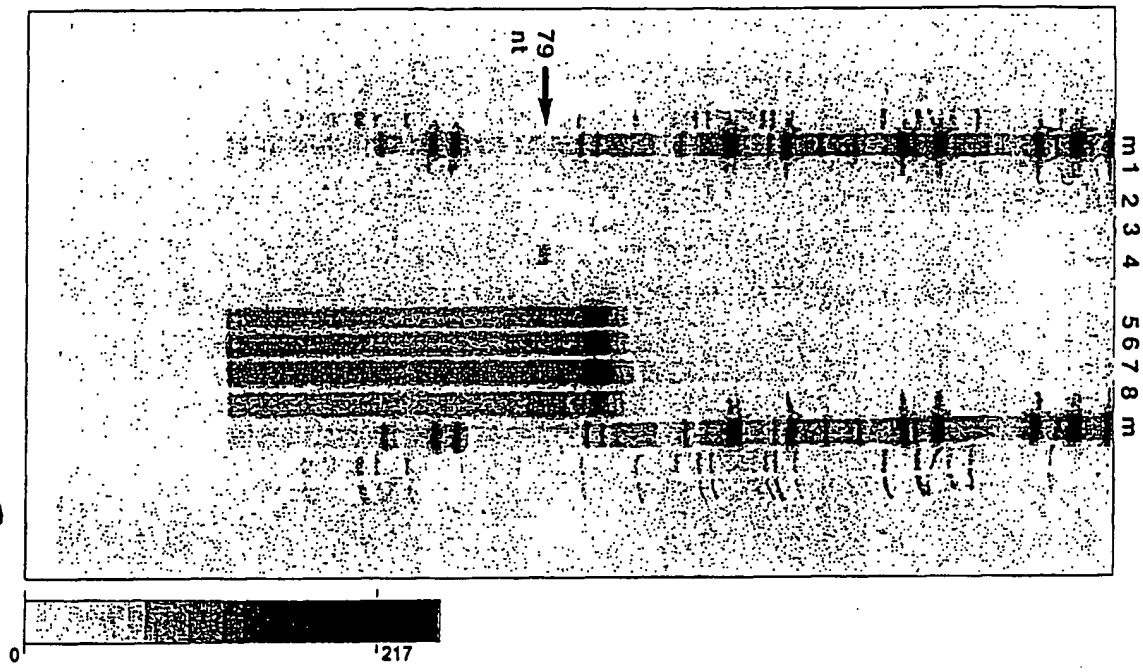


FIG 6 - Analysis of Expression from LXS-125 SDI-Infected Cells

Lane M - standard marker fragments

Lanes 1 & 5 - RNA from EJ cells

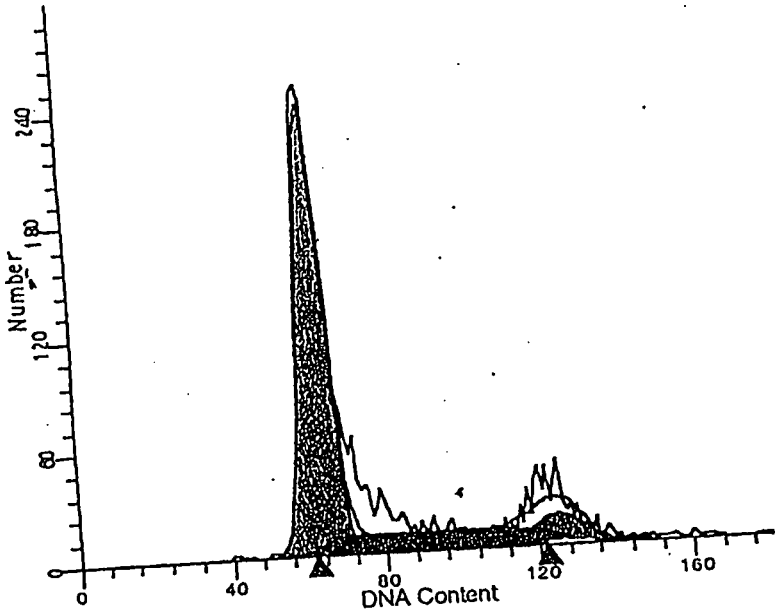
Lanes 2 & 6 - RNA from EJ cells treated with 10^{-6}M dexamethasone

Lanes 3 & 7 - RNA from EJ cell clone infected with LXS-125 SDI

Lanes 4 & 8 - RNA from EJ cell clone infected with LXS-125 SDI treated with 10^{-6}M dexamethasone

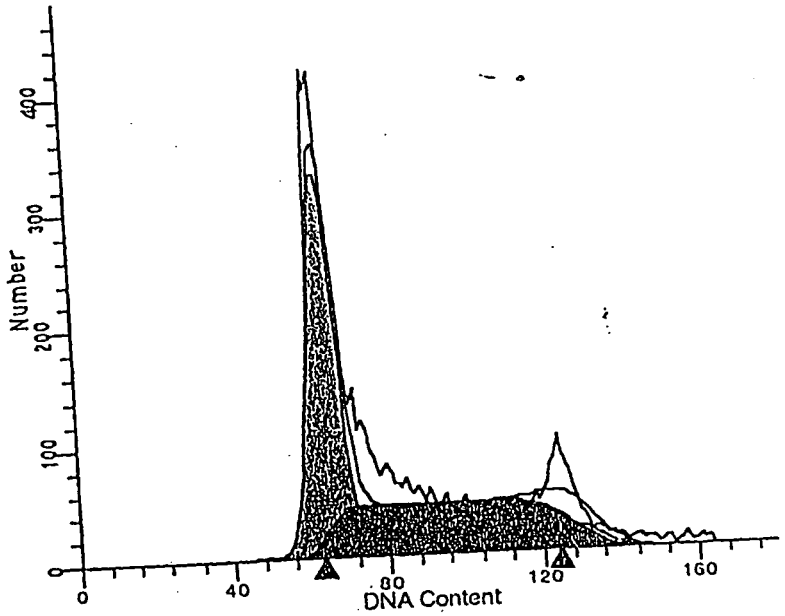
Lanes 1 - 4 using a probe specific for transcription from LXS-125 SDI

Lanes 5 - 8 using a probe specific for transcription from GAPDH



+DEX

	%
G_0G_1	66·8
G_2M	9·6
S	23·6



-DEX

	%
G_0G_1	48·2
G_2M	6·4
S	45·4

FIG 7

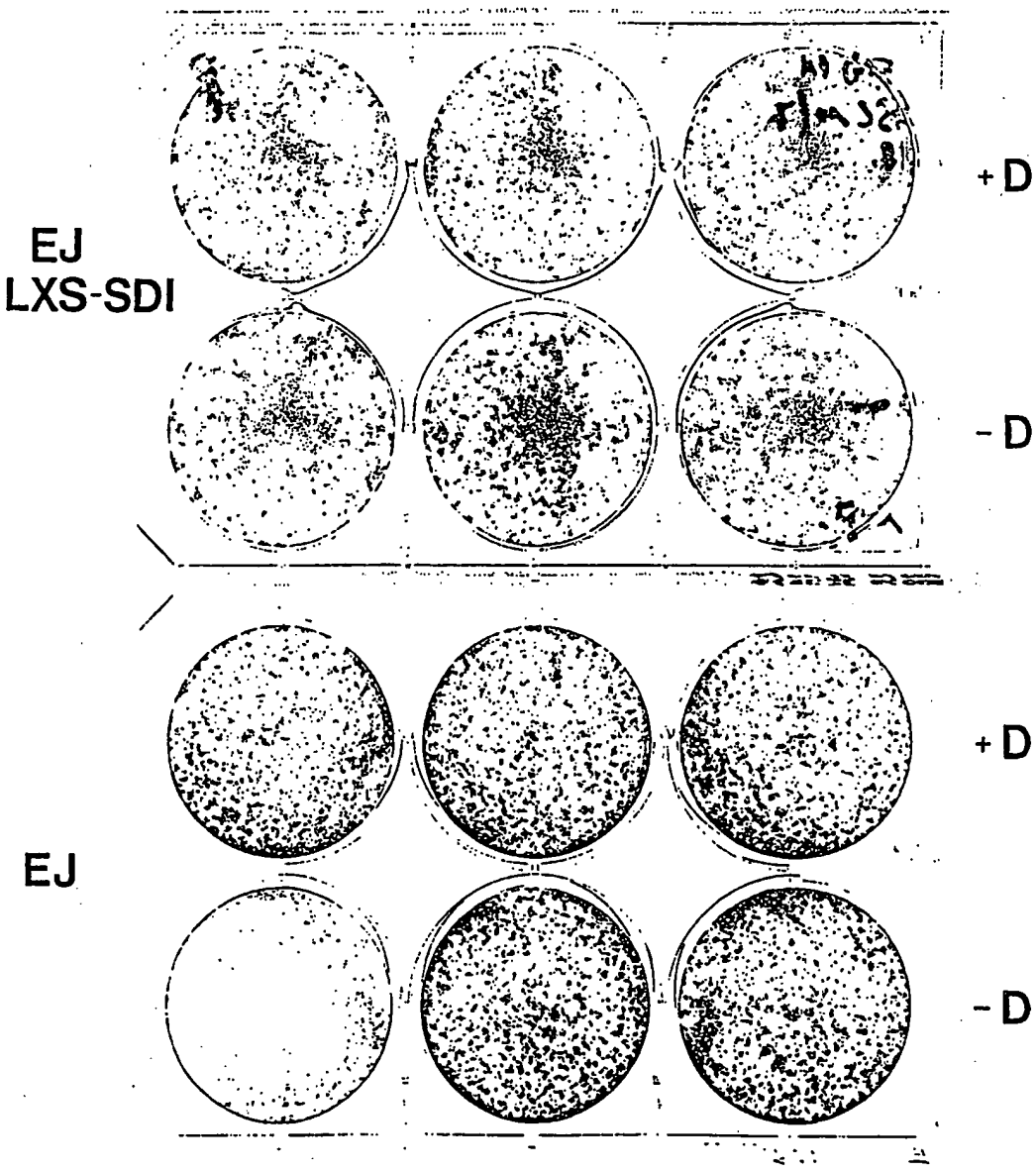


FIG 8